



**KPL**

Power Your  
Immunoassays

*See More  
SIGNAL.  
Less Noise.*

**Immunoassay Products**





## See More **SIGNAL**. Less Noise.

In nature many animals use camouflage to escape detection. An animal's survival often depends upon its ability to blend in with its environment, making it difficult to see. Just as in nature, your target protein can also be camouflaged in a complex "environment", making it difficult to see too. To create a successful immunoassay, you must be able to clearly distinguish your target from background. Kirkegaard & Perry Laboratories, Inc. (KPL) products improve immunoassays by increasing the level of specific signal while reducing unwanted background. Throughout the catalog, KPL uses a series of pictures of animals camouflaged in their environment to illustrate the benefit of KPL products: "See More SIGNAL. Less Noise." With KPL products, researchers are able to see more and, as a result, achieve more. *Power Your Immunoassays with KPL!*

### About KPL

For nearly 4 decades KPL has been a leader in developing and manufacturing high-quality immunochemicals for life science researchers and *in vitro* diagnostics. From our initial production of affinity purified secondary antibodies to our current offering of more than 800 reagents and complete assay kits, KPL has been here to serve your needs.

At KPL we know purity, sensitivity, and availability are important to you. That is why KPL only sells what it makes, and everything is made using an ISO 9001:2008 quality system. We also maintain virtually 100% in-stock availability for same-day shipment.

Whether our reagents are being used by a laboratory researcher or commercially by a leading diagnostic kit manufacturer, KPL always adheres to the highest quality standards. That is why with KPL products you see more signal and less noise.

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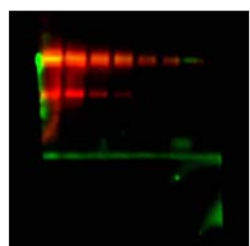
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910 Clopper Road  
Gaithersburg, MD 20878 USA  
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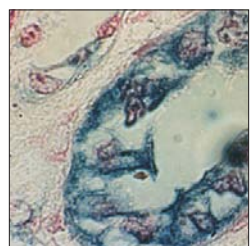
## Table of Contents



- 4**  
ELISA  
Kits and Reagents



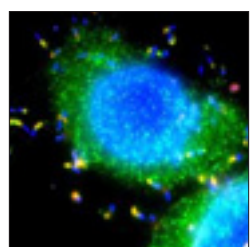
- 9**  
Western Blotting  
Kits and Reagents



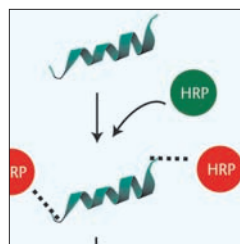
- 17**  
Immunohistology  
Kits and Reagents



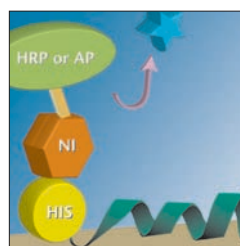
- 22**  
Secondary Antibodies  
and Conjugates



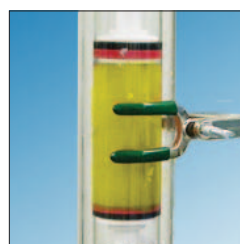
- 28**  
Anti-Bacteria Primary  
Antibodies and Controls



- 32**  
Protein Labeling  
Kits and Reagents



- 35**  
Recombinant Protein  
Detection and Analysis



- 39**  
Sample Preparation  
and Protein Purification



- 43**  
Immunoassay  
Support Reagents



- 48**  
Custom/Bulk  
Products and Services

Alphabetical Index ..... **51**

Sales Partners ..... **53**



See More with KPL ELISA Products

# ELISA *Kits and Reagents*

- ➔ **Substrates** for colorimetric or chemiluminescent detection
- ➔ **Support reagents** include blocking solutions, coating solutions, wash solutions, and stop solutions.
- ➔ Universal **ELISA kits** contain all the reagents required to perform an ELISA, except for primary antibodies.
- ➔ A comprehensive line of **secondary antibodies**. See Page 22 for more information.



# ELISA

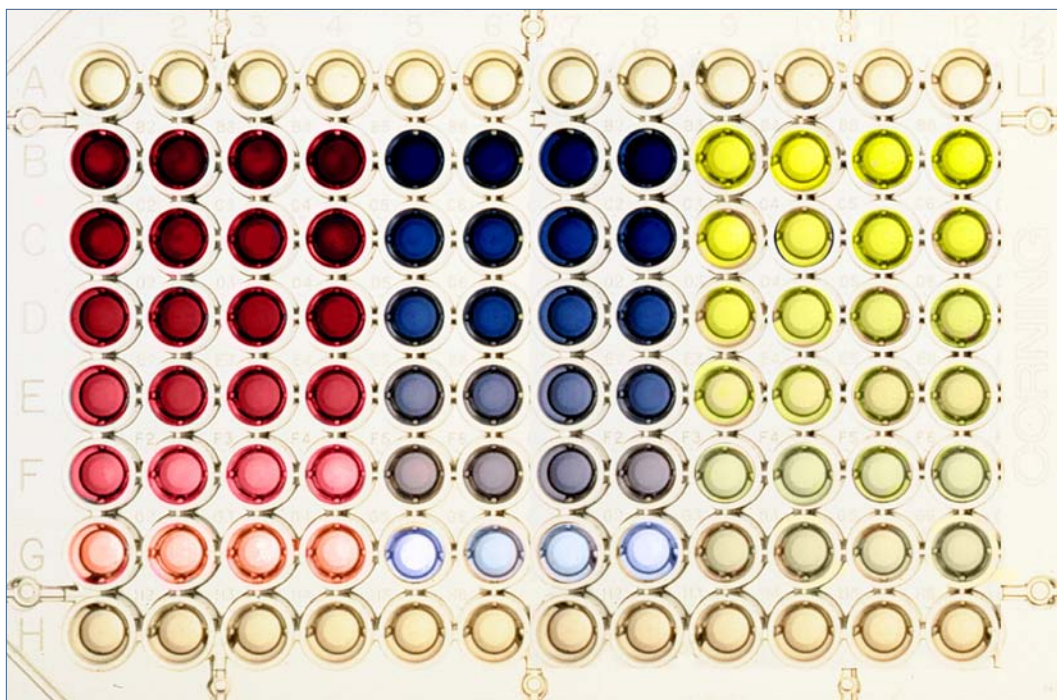
## *Kits and Reagents*

Enzyme-linked immunosorbent assay (ELISA) is one of the most common immunodetection techniques used to determine the presence of an antigen or antibody in a sample. KPL offered one of the first ready-to-use ELISA substrates in the 1980s and has been a leader in the field ever since.

Over time ELISA became popular because of its ease of use, high sensitivity, high-throughput capacity, accuracy, as well as the ready availability of high-quality reagents. In addition, it allows for rapid screening or quantitation of a large number of samples. Variations can be used to screen protein-protein, protein-nucleic acid, or nucleic acid-nucleic acid interactions in microarrays.

### **ELISA Substrates**

Substrates are the cornerstones for developing ELISAs with high sensitivity and low background. KPL makes a range of substrates that are sure to meet your needs for sensitivity, stability, and convenience.



*Both FirePhos and BluePhos can detect less than 0.5 pg of phosphatase and produce a significantly higher signal than pNPP. The color produced is stable and linear over a wide dynamic range. pNPP, although less sensitive, is a widely used substrate that offers reliable performance in many immunoassays. Whichever colorimetric substrate you choose, you will enjoy the benefits of excellent signal-to-noise with higher sensitivity and lower background than that of many other AP colorimetric microwell substrates on the market.*

## Choosing an ELISA Substrate

ELISA substrates differ in sensitivity, ease-of-use, stability, and compatibility with equipment. When choosing a substrate, consider all these criteria.

**Sensitivity** Select a substrate that will work at or slightly below the limits of detection required for a specific assay, so that less optimization is required. The more sensitive a substrate, the more difficult it is to optimize, since incomplete blocking and non-specific binding are more readily detected.

**Ease-of-use** Substrates require different procedures for preparing the working substrate solution. Some are ready-to-use formulations while others require mixing of two solutions immediately before use or adding tablets to a buffer solution. Ready-to-use substrates are easier to use and conducive to automated equipment.

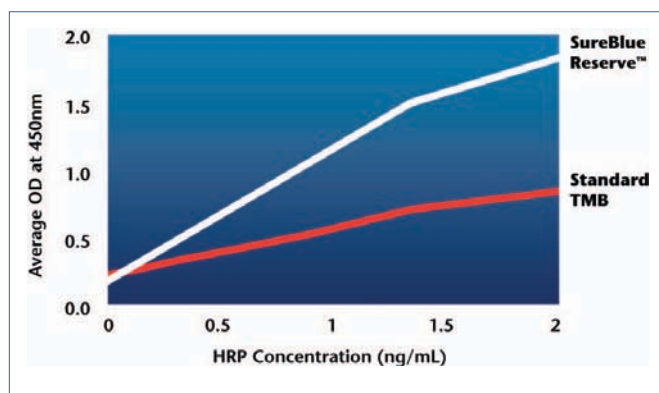
**Stability** In general, ready-to-use substrates are less stable than substrates where the active components remain separated until use. How quickly you will use the bottle of substrate is a key question.

**Equipment** Colorimetric substrates require a plate spectrophotometer (ELISA reader). Chemiluminescent substrates require a plate luminometer. Does your lab have the right equipment for your substrate choice?

## Our Offerings

### Colorimetric Substrates

In enzymatic reactions, colorimetric substrates produce a visible end product. KPL offers colorimetric substrates for both horseradish peroxidase (HRP) and alkaline phosphatase (AP) detection with several levels of sensitivity and speed of kinetics. KPL's substrates are unsurpassed in terms of stability and consistency. Results can be read with an ELISA reader or by the eye.



*Dilutions of HRP conjugate were added to microplate wells and reacted with either standard TMB or SureBlue Reserve. Reactions were stopped with TMB Stop Solution and read at 450 nm. The data demonstrates SureBlue Reserve's superior sensitivity.*

### TMB 2-Component Microwell Peroxidase Substrate Kit

3,3',5,5'-Tetramethylbenzidine (TMB) substrates produce a blue color read at 650 nm when reacting with HRP. TMB is very sensitive, but may also produce more background signal if blocking, washing, and antibody steps are not optimized. TMB is more quickly oxidized than other HRP substrates.

### SureBlue™ and SureBlue Reserve™ TMB

SureBlue and SureBlue Reserve TMB substrates produce a deep blue soluble product when reacted with HRP-labeled antibodies. Both substrates are offered as fast-acting, ready-to-use solutions with excellent signal-to-noise and consistent performance, minimizing the need for re-optimization from one lot to another. SureBlue Reserve is the substrate of choice when highest sensitivity is required, enabling detection of low levels of target protein.

### ABTS® 1-Component Microwell Peroxidase Substrate Kit

2,2'-Azino-di-(3-ethylbenzthiazoline-6-sulfonate) (ABTS) substrates produce a blue-green color read at 405 nm when reacting with HRP. ABTS is less sensitive than

TMB, and color development is slower, providing a broader detection range. ABTS substrates are extremely stable. KPL offers a ready-to-use solution or a two-component system.

### pNPP Microwell Substrate System

p-Nitrophenylphosphate (pNPP) develops a soluble yellow color when it reacts with AP. The yellow color is measurable between 405 and 410 nm. Since it is economical and reliable, pNPP is widely used. Working substrate is made by mixing pNPP tablet with DEA buffer and water.

### BluePhos® Microwell Substrate Kit/ FirePhos™ Microwell Phosphatase Substrate System

BluePhos and FirePhos are soluble, proprietary forms of 5-bromo-4-chloro-3-indolyl phosphate (BCIP) that react with AP. They feature greater sensitivity, lower background, and more convenience than pNPP. BluePhos is read at 620 nm, while FirePhos is read at 550 nm. Both are two-component formats, simply mix and use.

### Stopping Solutions

KPL manufactures stop solutions for all our colorimetric substrates. See page 46 in the Support Reagents section.

	CATALOG NO.*	ENZYME	SENSITIVITY	FORMAT	STOP SOLUTION
<b>SureBlue Reserve TMB 1-Component Microwell Peroxidase Substrate</b>	53-00-01	HRP	Low Picogram	Ready-to-use	TMB Stop TMB BlueStop
<b>SureBlue TMB 1-Component Microwell Peroxidase Substrate</b>	52-00-01	HRP	High Picogram	Ready-to-use	TMB Stop TMB BlueStop
<b>TMB 2-Component Microwell Peroxidase Substrate</b>	50-76-11	HRP	Picogram	2-component	TMB Stop TMB BlueStop
<b>BluePhos Microwell Substrate</b>	50-88-02	AP	Low Nanogram	2-component	APStop
<b>FirePhos Microwell Phosphatase Substrate System</b>	50-87-10	AP	Low Nanogram	2-component	APStop
<b>ABTS 1-Component Microwell Peroxidase Substrate</b>	50-66-18	HRP	Nanogram	Ready-to-use	ABTS Stop
<b>ABTS 2-Component Microwell Peroxidase Substrate</b>	50-62-00	HRP	Nanogram	2-component	ABTS Stop
<b>pNPP Microwell Substrate System</b>	50-80-00	AP	Nanogram	Tablet and buffer	APStop

\* Smallest catalog size is listed. Other sizes are available.

## Chemiluminescent Substrates

Microplate-based chemiluminescence ELISA systems are becoming popular due to increased sensitivity over conventional colorimetric methods, reaction speed, and simplified procedures (no stopping reagent required). Results must be read with a luminometer. Special white or black microplates are required.

### LumiGLO® Peroxidase Chemiluminescent Substrate

This luminol-based substrate reacts with HRP and is easily optimized to detect with greater sensitivity than entry-level colorimetric substrates. LumiGLO produces rapid light emission with 5- to 30-minute signal stability, depending on HRP concentration.

### LumiGLO Reserve™ Chemiluminescent Substrate

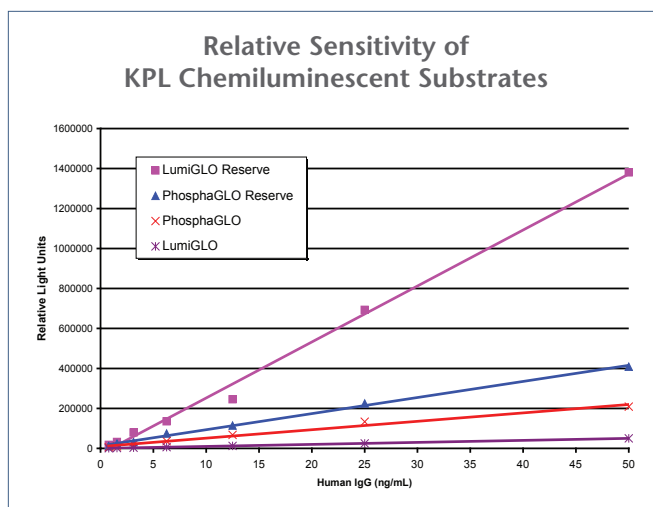
This enhanced luminol substrate reacts with HRP and is one of the most sensitive substrates available for ELISA applications. When properly optimized, it is more sensitive than colorimetric substrates.

### PhosphaGLO™ Phosphatase Substrate

This dioxetane-based substrate reacts with AP. When properly optimized, it is more sensitive than colorimetric AP substrates. Ready-to-use formulation is extremely stable.

### PhosphaGLO Reserve™ Phosphatase Substrate

This enhanced dioxetane-based substrate reacts with AP and is the most sensitive AP substrate available for ELISA. Ready-to-use formulation is extremely stable.



Directly detected Chemi-ELISA (black plates) of purified Human IgG using HRP or AP labeled Goat anti-Human antibody (500 ng/mL). HRP substrates were measured after a 1 min incubation of substrate and AP conjugates were measured after 15 min.

## ELISA Support Reagents

KPL provides a broad range of support reagents, including blockers, wash solutions, substrate stopping solutions, and accessories that reduce background and improve reproducibility. See page 43 in the **Support Reagents** section.

	CATALOG NO.*	ENZYME	SENSITIVITY	FORMAT
<b>LumiGLO Reserve Chemiluminescent Substrate</b>	54-71-01	HRP	Femtogram	2-component
<b>LumiGLO Peroxidase Chemiluminescent Substrate</b>	54-61-02	HRP	Low Nanogram	2-component
<b>PhosphaGLO Reserve Phosphatase Substrate</b>	55-60-01	AP	High Femtogram	Ready-to-use
<b>PhosphaGLO Phosphatase Substrate</b>	55-60-03	AP	Low Picogram	Ready-to-use

\* Smallest catalog size is listed. Other sizes are available.





See More with KPL Western Blot Products

# Western Blotting *Kits and Reagents*

- ➔ **Universal Western blot kits** contain essential reagents to perform a Western blot.
- ➔ **Substrates** for colorimetric or chemiluminescent detection
- ➔ **Support reagents** includes blocking solutions, buffers, and wash solutions.
- ➔ A comprehensive line of **secondary antibodies** – see page 22 for more information

# Western Blotting

## *Kits and Reagents*

Western blotting is one of the most popular immunodetection techniques utilized by researchers for protein detection. The technique first separates proteins by molecular weight and then uses specific antibodies and conjugates to visualize proteins of interest. As researchers looked for non-radioactive means to measure immunodetection, KPL stayed versatile and continuously invested in expansion of our offering to support chemiluminescent, colorimetric, and fluorescent detection techniques.

### **Chemiluminescence, Colorimetric, or Fluorescence for Western Blot Detection**

Historically, colorimetric detection and low-sensitivity chemiluminescent detection have enabled the expansion of research. Recent advances in chemiluminescent molecule design have coincided with the increased need for sensitivity and the development of image analysis systems. Fluorescent detection offers significant step reduction and multiplexing with sensitivity comparable to colorimetric reagents.

### **Kits or Components**

Time spent on optimizing the performance of Western blots has always been a problem. KPL has two solutions to this problem: highly optimized kits and individual components. Whether you choose the convenience of kits or prefer building your own system, KPL has the Western blot products for you.

## *Our Offerings*

### **Chemiluminescent Blotting Kits**

KPL's line of chemiluminescent Western blot kits provides the most sensitive detection solutions. Our offering includes the highly sensitive SignalOCK™ kits optimized for rapid results and our value-conscious Protein Detector™ kit for routine detection. All kits offer comprehensive protocols and can be used with both nitrocellulose and PVDF membranes.

#### **SignalOCK™ HRP ChemiWestern Kit (Imager)**

This kit is optimized for rapid, one-minute image analysis with a high signal and very low background, so you spend less time optimizing and more time analyzing results. Composed of LumiGLO Ultra™ substrate, SignalOCK Blocking Solution, and a wash solution, the kit is optimized for an imager, has femtogram level of sensitivity detection, and enough reagents to process ten 10 x 10 cm<sup>2</sup> blots.

#### **SignalOCK™ HRP ChemiWestern Kit (Film)**

When you need a kit optimized for film that will give you femtogram levels of sensitivity with a very high signal and low background, this is the kit to choose. The kit contains LumiGLO Reserve™ substrate, SignalOCK Blocking Solution, and a wash solution, all of which will process ten 10 x 10 cm<sup>2</sup> blots.



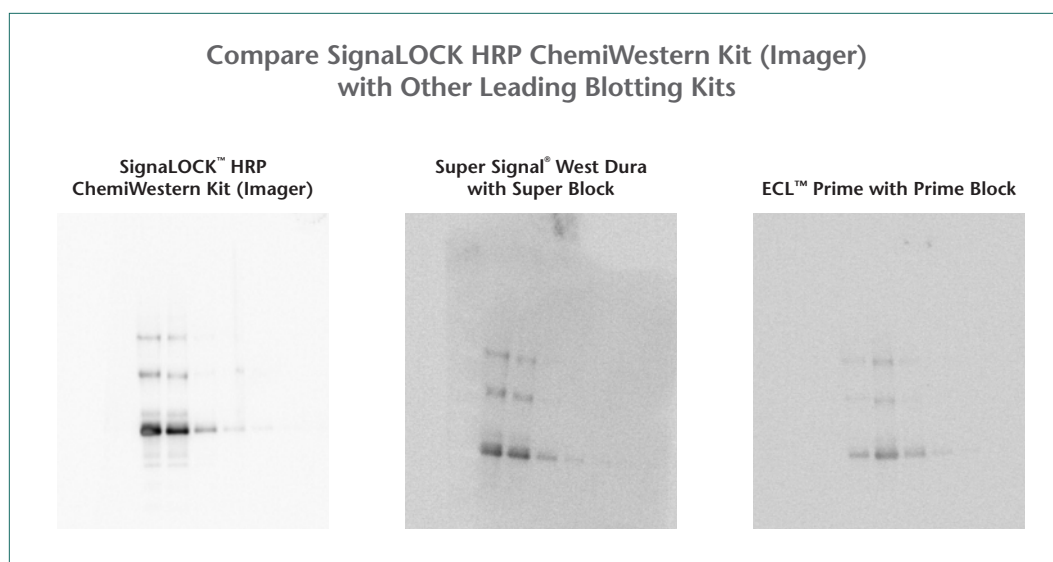
### SignalLOCK™ AP ChemiWestern Kit (Film/Imager)

Suitable for film or imager, this kit will produce a stable signal for days so you have greater flexibility in controlling the assay and generation of multiple image exposures at femtogram levels of sensitivity. The kit contains enough PhosphaGLO Reserve substrate, SignalLOCK Blocking Solution, and wash concentrate for ten 10 x 10 cm<sup>2</sup> blots.

### Protein Detector™ LumiGLO® Western Blot Kit

This kit is recommended for routine protein detection challenges enabling low picogram detection. The ready-to-use reagents enable convenient and quick assay preparation. The kit includes LumiGLO® substrate, KPL's Detector™ Block, a wash solution, and for added flexibility, affinity purified anti-mouse and anti-rabbit secondary antibodies in stabilized, liquid form for quick dilution. There are enough reagents in the kit to process 25 x 10 cm<sup>2</sup> blots.

*Comparison of nanogram sensitivity using SignalLOCK HRP ChemiWestern Kit (Imager), Super Signal West Dura, and ECL Prime and their recommended blockers. Membranes were blocked overnight and protein detected using HRP-labeled anti-human IgG (γ). Immunoblots were imaged for 1 minute after application of substrate.*



*Super Signal is a registered trademark of Thermo Fisher Scientific, Inc. and its subsidiaries.  
ECL and Prime are trademarks of GE Healthcare.*

## Colorimetric Blotting Kits

Colorimetric detection offers a reliable means for analyzing proteins on membranes, when the sensitivity of chemiluminescent substrates is not required. KPL's universal Protein Detector™ Colorimetric Western blot kits offer a choice of HRP- or AP-labeled secondary antibodies, wash, Detector™ Block, and a corresponding colorimetric substrate, TMB or BCIP/NBT. The kits have everything you need except primary antibody and are optimized to produce sharp band resolution with minimal background staining.

### Protein Detector™ TMB Western Blot Kit

TMB is the most sensitive colorimetric blotting substrate for use with HRP. It produces a localized, blue precipitate with high signal-to-noise and minimal background.

### Protein Detector™ BCIP/NBT Western Blot Kit

BCIP/NBT is the most sensitive colorimetric substrate for use with AP. It produces a dark purple precipitate with sharp band resolution and little background staining.



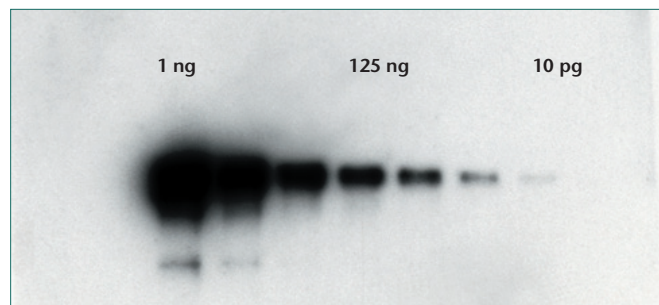
## Chemiluminescent and Colorimetric Western Blotting Kits Comparison and Selection Guide

DETECTION KITS*	CATALOG NO.	ENZYME	KIT COMPONENTS	SENSITIVITY	NUMBER OF BLOTS PER KIT
<b>SignalLOCK™ HRP ChemiWestern Kit (Imager)</b>	54-54-00	HRP	LumiGLO Ultra Substrate, SignalLock Block, wash solution	Low Femtogram	10
<b>SignalLOCK™ HRP ChemiWestern Kit (Film)</b>	54-53-00	HRP	LumiGLO Reserve Substrate, SignalLock Block, wash solution	Low Femtogram	10
<b>Protein Detector™ LumiGLO Western Blot Kit</b>	54-12-50	HRP	LumiGLO Substrate, Detector Block, wash solution, HRP conjugate	Low Picogram	25
<b>SignalLOCK™ AP ChemiWestern Kit (Film/Imager)</b>	54-56-00	AP	PhosphaGLO Reserve Substrate, SignalLock Block, wash solution	High Femtogram	10
<b>Protein Detector TMB Western Blotting Kit</b>	54-11-50	HRP	TMB Substrate, Detector™ Block, wash solution, HRP conjugates	Picogram	25
<b>Protein Detector BCIP/NBT Western Blotting Kit</b>	55-11-50	AP	BCIP/NBT Substrate, Detector Block, wash solution, AP conjugates	Picogram	25

\* Kits have a minimum 12-month stability.

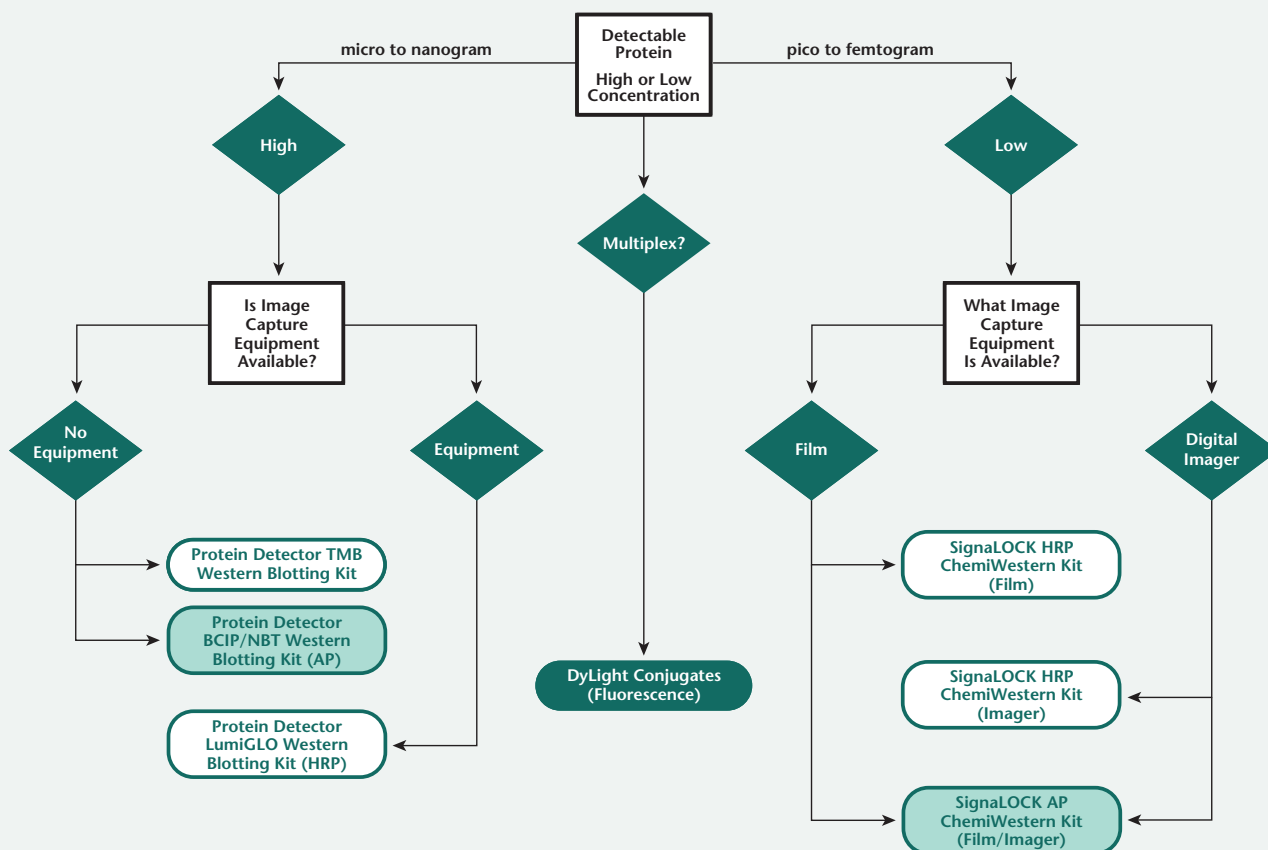
## Western Blotting Substrates

High substrate quality is imperative when developing Western blots with high sensitivity and low background. KPL's line of colorimetric and chemiluminescent substrates are the cornerstone of our product offering of Western blotting kits and reagents. They differ in sensitivity, ease-of-use, stability, visualization techniques, and price.



Two-fold serial dilutions of Mouse IgG (1 ng – 16 pg) were separated by SDS-PAGE, transferred to PVDF and detected using HRP-labeled anti-mouse antibody. Film was exposed for 10 minutes and analyzed for sensitivity and signal to noise.

## What Detection System Should I Use?



## Chemiluminescent Substrates

Chemiluminescence offers the best choice for detecting minute amounts of protein. Chemiluminescent substrates emit light when catalyzed with the appropriate enzyme conjugated to an antibody. All chemiluminescent blots emit photons over time, thus requiring either a darkroom and X-ray film or an imaging system to capture results.

### LumiGLO Ultra™ Chemiluminescent Substrate

LumiGLO Ultra reacts with HRP and produces an intense signal for hours with extremely low background. It is optimized for use with image analysis systems and is ideal for detecting poorly expressed proteins. Images may be obtained with only a one-minute exposure after substrate application, depending on the amount of protein present.

### LumiGLO Reserve™ Chemiluminescent Substrate

LumiGLO Reserve reacts with HRP and is one of the most sensitive substrates available for Western blotting applications. It has a wide dynamic range that is linear for a long period of time. Optimized for film analysis, LumiGLO Reserve enables exposures up to 8 hours after substrate application, depending on the amount of protein present.

### LumiGLO® Chemiluminescent Substrate

LumiGLO provides picogram detection of HRP-labeled conjugates and is ideal for routine blotting applications. LumiGLO provides increased sensitivity over colorimetric substrates and allows a permanent record of results on X-ray film.

### PhosphaGLO Reserve™ Chemiluminescent Substrate

PhosphaGLO Reserve reacts with AP and is ideal for detection of proteins expressed in femtogram concentrations. It produces a sustained signal for days, enabling generation of multiple image exposures.

### PhosphaGLO™ Chemiluminescent Substrate

PhosphaGLO provides picogram detection of AP-labeled conjugates and is ideal for routine blotting applications. Superior glow time (3 – 5 days) allows generation of multiple exposures.

Chemiluminescent Substrates Comparison and Selection Guide

SUBSTRATE	CATALOG NO.*	ENZYME	FORMAT	SENSITIVITY	NUMBER OF BLOTS PER ITEM
LumiGLO Ultra Chemiluminescent Substrate	54-51-00	HRP	2-component	Femtogram	10
LumiGLO Reserve Chemiluminescent Substrate	54-71-01	HRP	2-component substrate, wash solution	Femtogram	6
LumiGLO Peroxidase Chemiluminescent Substrate	54-61-02	HRP	2-component	Picogram	6
PhosphaGLO Reserve Chemiluminescent Substrate	55-60-01	AP	Ready-to-use	Femtogram	6
PhosphaGLO Chemiluminescent Substrate	55-60-03	AP	Ready-to-use	Picogram	6

\* Smallest catalog size is listed. Each substrate is available in several sizes. Substrates stable for a minimum of one year.



## Colorimetric Substrates

Colorimetric substrates utilize an enzymatic reaction to produce a visible end product. They are ideal when there is a large amount of target protein. Results can be read on the bench top and photographed for longevity. No darkroom or imaging system is necessary.

KPL offers colorimetric substrates for both HRP and AP detection with several levels of sensitivity. They are unsurpassed in terms of stability and consistency.

### TMB 1-Component Membrane Peroxidase Substrate

TMB Membrane Substrate produces a bright blue precipitate at the reaction site in the presence of HRP. It is the most sensitive substrate for colorimetric blotting applications.

### 4CN 2-Component Membrane Peroxidase Substrate

4CN Substrate produces a purple precipitate at the reaction site in the presence of HRP. It provides an effective alternative to more sensitive substrates when background is a problem.

### BCIP/NBT 1-Component Phosphatase Substrate

BCIP/NBT substrate produces a deep purple precipitate at the reaction site in the presence of AP. In contrast to other colorimetric membrane substrates, it produces a permanent stain.

### FirePhos™ Membrane Phosphatase Substrate

FirePhos Substrate produces a bright red precipitate at the reaction site in the presence of AP. The substrate is linear over a wide dynamic range and resists fading when exposed to light.

Color Substrates Comparison and Selection Guide

SUBSTRATE	CATALOG NO.*	ENZYME	FORMAT	SENSITIVITY	NUMBER OF BLOTS PER ITEM
TMB 1-Component Membrane Peroxidase Substrate**	50-77-18	HRP	Ready-to-use	Picogram	10
4CN 2-Component Membrane Peroxidase Substrate	50-73-00	HRP	2-component	Nanogram	60
BCIP/NBT 1-Component Phosphatase Substrate**	50-81-18	AP	Ready-to-use	Low Nanogram	10
FirePhos Membrane Phosphatase Substrate	50-81-30	AP	Ready-to-use	Low Nanogram	10

\* Smallest catalog size is listed. Each substrate is available in several sizes. Substrates stable for a minimum of one year.

\*\* Product also offered as more stable three-component system.

## Fluorescent Western Blotting

When two or more antigens need to be probed simultaneously, fluorescent Western blots are utilized and easily performed with one or more of KPL's fluorescent-labeled conjugates. An excitation wavelength (specific to the fluorophore) is exposed to the conjugate, and a corresponding emission wavelength is measured to determine the amount of signal present. The spectral separation of their absorption and emission profiles facilitates the design of multiple labeling experiments.

KPL's DyLight™ and Cy™ Dye conjugates combine the sensitivity and reproducibility of our affinity purified secondary antibodies with a series of fluorescent dyes that span the light spectrum from visible to infrared. They are especially recommended for Western blotting. See chart below for KPL's range of products with catalog numbers.

FLUOROPHORE	COLOR	EX/EM*	REPLACES	CATALOG NO.		
				ANTI-HUMAN IgG	ANTI-MOUSE IgG	ANTI-RABBIT IgG
DyLight 405	Blue	400/420	Alexa 405 and Cascade Blue	072-08-10-06	072-08-18-06	072-08-15-06
DyLight 488	Green	493/518	Alexa 488, FITC	072-03-10-06	072-03-18-06	072-03-15-06
DyLight 550	Yellow	550/568	Alexa 546, Alexa 555, TRITC, Cy3	←	Coming soon!	→
DyLight 594	Orange	593/618	Alexa 594, Texas Red	072-09-10-06	072-09-18-06	072-09-15-06
DyLight 633	Red	638/658	Alexa 633	072-10-10-06	072-10-18-06	072-10-15-06
DyLight 650	Red	646/674	Alexa 647, Cy5	←	Coming soon!	→
DyLight 680	Near Infrared	682/715	Alexa 680, Cy5.5	072-06-10-06	072-06-18-06	072-06-15-06
DyLight 800	Infrared	770/794	IRDye 800	072-07-10-06	072-07-18-06	072-07-15-06
Cy3	Orange-Red	550/570	Alexa 546	072-01-10-06	072-01-18-06	072-01-15-06
Cy5	Red	650/670	Alexa 647	072-02-10-06	072-02-18-06	072-02-15-06

\* Excitation and emission spectra in nanometers

## Support Reagents

KPL provides a broad range of support reagents, including blocking solutions, wash solutions, and buffers that reduce background and improve reproducibility. See page 43 in the Support Reagents section.



See More with KPL HistoMark® Products

# Immunohistology *Kits and Reagents*

- ➔ **Biotin-Streptavidin Kits** provide high sensitivity and low background, utilizing minute amounts of antibody.
- ➔ Stable liquid **Staining Systems** reduce preparation time and eliminate waste.
- ➔ **Counterstains** provide sharp contrast and clear nuclear detail.
- ➔ **Support Reagents** include mounting media, enzyme and serum blocks, and wash solutions.
- ➔ **Secondary Antibodies** are labeled with enzymes and fluorophores. See Antibodies and Conjugates on page 22.



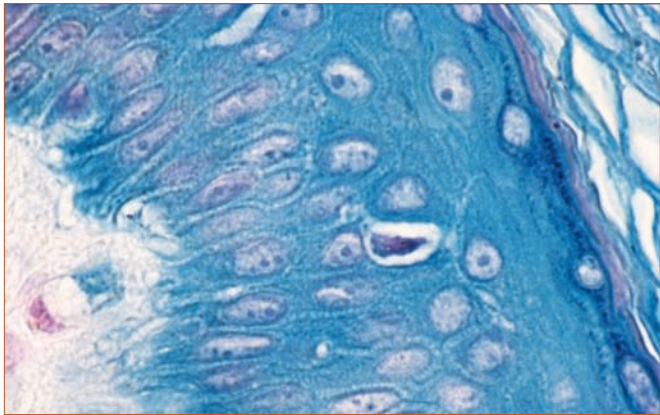
# Immunohistology

## Kits and Reagents

Immunohistochemistry (IHC) is a powerful technique that allows researchers to view tissue architecture, and detect the distribution and localization of antigens in specific cells via antibody binding with colorimetric or fluorescent methods. KPL has developed a variety of biotin/streptavidin kits and colorimetric staining systems for HRP, AP, and  $\beta$ -galactosidase detection that provide rapid, precise localization of antigens in cells and tissues. They facilitate multiple staining applications and are optimized for maximum sensitivity and convenience.

### IHC Detection Methods

The optimal detection method depends on the level of target antigen and the demands of the assay. The following chart presents the features of several common detection methods to be considered. Whatever method you choose, KPL has a range of products that facilitate accurate and sensitive staining.



*HistoMark X-Gal demonstrates keratin in skin.*

FEATURES	DIRECT	INDIRECT	BIOTIN-STREPTAVIDIN
Number of Steps	1 Step	2 Steps	3 Steps
Signal Amplification	No amplification	Moderate to good	High
Advantages	Simple, rapid	Few secondary antibodies required to accommodate many assays (labeled anti-mouse, anti-rabbit, etc.)	<ul style="list-style-type: none"><li>• Streptavidin conjugate can detect any biotinylated protein.</li><li>• Each biotin can bind 4 molecules of labeled streptavidin enabling signal amplification</li><li>• High affinity association of biotin to streptavidin permits extensive washing for low background</li></ul>
Disadvantages	Signal may be difficult to detect	Endogenous enzyme may cause background issues	Endogenous enzyme or biotin may cause background issues
Best for	Abundant targets; cell surface antigens	Target that needs slight amplification for visualization	Rare biomarkers; low abundance targets

## Detection Labels – Colorimetric and Fluorescent Staining

In colorimetric detection an enzyme conjugated to an antibody is reacted with a substrate to yield an intensely colored product that can be analyzed with light microscopy. Alternatively, the primary or secondary antibody can be tagged with a fluorophore, such as fluorescein, rhodamine, Cy Dyes, or DyLight dyes and detected directly with a fluorescence microscope.

DETECTION METHOD	SUBSTRATES AVAILABLE	MULTIPLEX STAINING	BACKGROUND ISSUES	PERMANENT STAIN	TISSUE MORPHOLOGY	EQUIPMENT REQUIREMENTS
Colorimetric HRP/AP	Variety of color combinations	Double labeling	Endogenous enzyme	Varies with stain	Visible with counterstain	Light microscope
Fluorescent	N/A	Multiple labeling	Auto-fluorescence	Will fade: need to photograph	Varies with technique	Fluorescence microscope

## Our Offerings

### HistoMark® Biotin-Streptavidin Kits

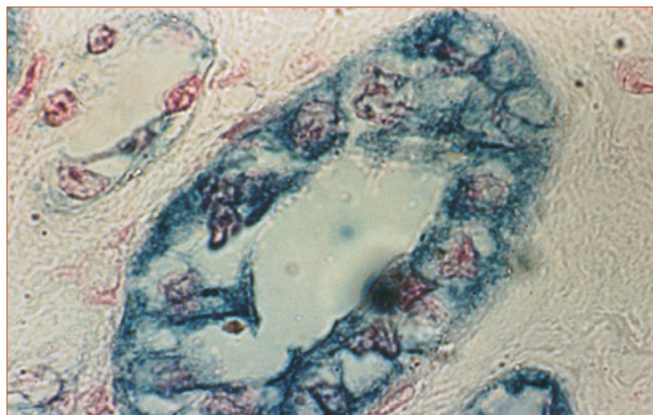
HistoMark Biotin-Streptavidin Kits are optimized for maximum sensitivity and convenience. KPL offers a selection of biotin-streptavidin kits labeled with peroxidase or phosphatase for use with primary antibodies produced in mouse, rabbit, rat, or goat. IHC staining is often prone to strong background staining, weak target antigen staining, and antibody cross-reactivity. KPL's HistoMark Biotin-Streptavidin Kits address these concerns by adsorbing biotinylated antibodies (mouse and rabbit) against human serum and anti-rat antibody against mouse serum. In addition, the use of serum block in the kit reduces non-specific binding of the secondary antibody.

HistoMark Peroxidase Biotin-Streptavidin Kits			HistoMark Phosphatase Biotin-Streptavidin Kits		
DESCRIPTION	CATALOG NO.	SIZE	DESCRIPTION	CATALOG NO.	SIZE
Mouse primary antibody	71-00-18	500 slides	Mouse primary antibody	71-00-39	500 slides
Rabbit primary antibody	71-00-19	500 Slides	Rabbit primary antibody	71-00-40	500 Slides
Rat primary antibody	71-00-20	500 Slides	Rat primary antibody	71-00-41	500 Slides
Goat primary antibody	71-00-26	500 Slides			

*Kit contents:* Biotinylated Secondary Antibody, HRP- or AP-labeled Streptavidin, Serum Block.

## HistoMark Immunohistochemical Staining Systems

KPL offers stand-alone substrates and a line of staining systems for the detection of peroxidase, phosphatase, and  $\beta$ -galactosidase conjugates. Visualization is based on enzymatic conversion of colorimetric substrates into a stable precipitate. These stains offer a variety of contrasting colors for multiple staining applications.



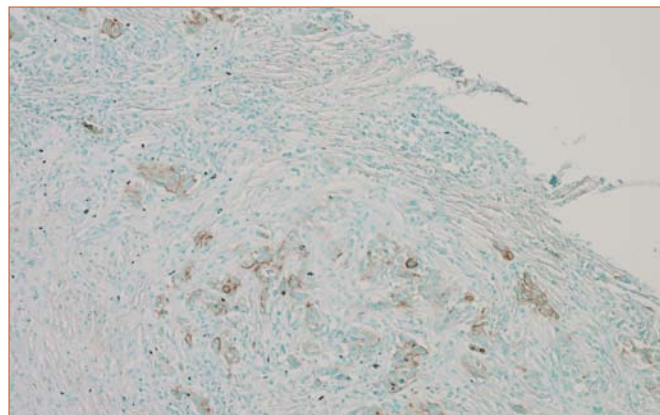
*HistoMark TrueBlue demonstrates epithelial membrane antigen in kidney.*

SUBSTRATE STAINING SYSTEMS	CATALOG NO.	ENZYME	SIZE	FORMAT	COLOR	SOLUBILITY	KEY FEATURES
<b>HistoMark TrueBlue</b>	54-78-00	HRP	500 slides	Ready-to-use	Blue	Alcohol or xylene insoluble	50 – 100 times more sensitive than DAB; non-carcinogenic
<b>DAB Reagent Set</b>	54-10-00	HRP	500 slides	3-component liquid	Brown	Alcohol or xylene insoluble	Can be made electron-dense
<b>StableDAB</b>	54-11-00	HRP	500 slides	2-component liquid	Brown	Alcohol or xylene insoluble	Stable for 2 weeks at 2 – 8° C after mixing
<b>HistoMark BLACK</b>	54-75-00	HRP	1000 slides	3-component liquid	Black	Alcohol or xylene insoluble	Enhanced DAB provides high sensitivity
<b>HistoMark ORANGE</b>	54-74-00	HRP	1000 slides	3-component liquid	Red-Orange	Alcohol or xylene insoluble	Enhanced DAB provides high sensitivity
<b>HistoMark RED</b>	55-69-00	AP	1000 slides	3-component liquid	Red	Alcohol or xylene insoluble	Contains levamisole to inhibit endogenous AP
<b>HistoMark BLUE</b>	55-70-00	AP	1000 slides	3-component liquid	Blue	Alcohol or xylene soluble	Contains levamisole to inhibit endogenous AP
<b>HistoMark X-Gal</b>	54-13-00	$\beta$ -Gal	200 slides	2-component liquid	Aqua	Alcohol or xylene insoluble	No need to block endogenous HRP and AP enzyme



## Counterstains

KPL's selection of counterstains enables visualization of cell nuclei and interpretation of tissue morphology. They provide sharp contrast with the principal stain and improve nuclear detail. Several counterstains can be used with multiple substrates as seen in the chart below.



*Breast tissue section demonstrating detection of Epithelial Membrane Antigen using DAB and Contrast Green counterstain.*

COUNTERSTAIN	CATALOG NO.	SIZE	STAIN	COLOR	TYPE OF STAIN	RECOMMENDED SUBSTRATE
Contrast GREEN	71-00-11	50 mL	Methyl green	Green	Nuclear	DAB, HistoMark BLACK or ORANGE
Contrast RED	71-00-05	50 mL	Nuclear fast red	Pink-red	Nuclear	HistoMark BLUE and other blue substrates
Contrast BLUE	71-00-06	50 mL	Hematoxylin	Blue	Nuclear	HistoMark RED and other red substrates
Orcein	71-01-01	50 mL	Orcein	Pink-red	Nuclear	HistoMark BLUE and other blue substrates
Propidium Iodide	71-04-01	1.0 mg	Propidium iodide	Red-fluorescent	Nuclear	FITC, Cy2, DyLight™ 488
DAPI	71-03-01	1.0 mg	DAPI	Blue-fluorescent	Nuclear	TRITC, Cy3, DyLight 549



Please visit [www.kpl.com/ihc](http://www.kpl.com/ihc) for our complete line of products, latest pricing, and easy ordering information.



See More with KPL Antibodies

# Secondary Antibodies and Conjugates

- ➔ More than **600 antibodies** to immunoglobulins of 20 animal species labeled with 18 tags
- ➔ Virtually 100% availability for **same-day shipment**

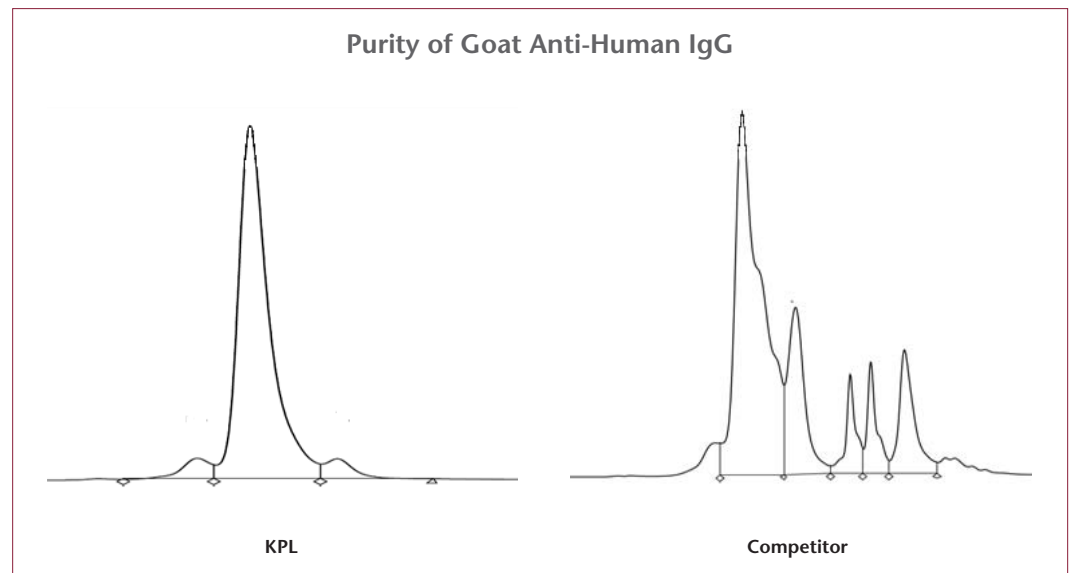
# Secondary Antibodies and Conjugates

Immunoassay techniques may differ, but the essential component that determines the level of sensitivity and specificity of the immunoassay is the antibody. KPL's affinity purification process yields reliable antibodies with a high degree of sensitivity and specificity, and are, therefore, ideal for use in immunoassay applications such as ELISA, Western blotting, and immunohistochemistry.

Affinity purified antibodies are especially valuable in immunoassays where extraneous serum proteins and unknown antibodies can interfere with test results. KPL's purification technology reduces nonspecific antibodies and cross-reactivity. When it comes to performance, KPL antibodies are the industry benchmark. Each antibody is produced with exacting standards for purity, sensitivity, specificity, and lot-to-lot consistency.

## Purity

KPL monitors the purity of its antibodies using size exclusion HPLC prior to affinity purification. The final product is virtually 100% pure – so pure they are sold by weight.



*HPLC results for Goat Anti-Human IgG demonstrates the purity of the KPL antibody vs. that of a leading competitor.*



High Sensitivity

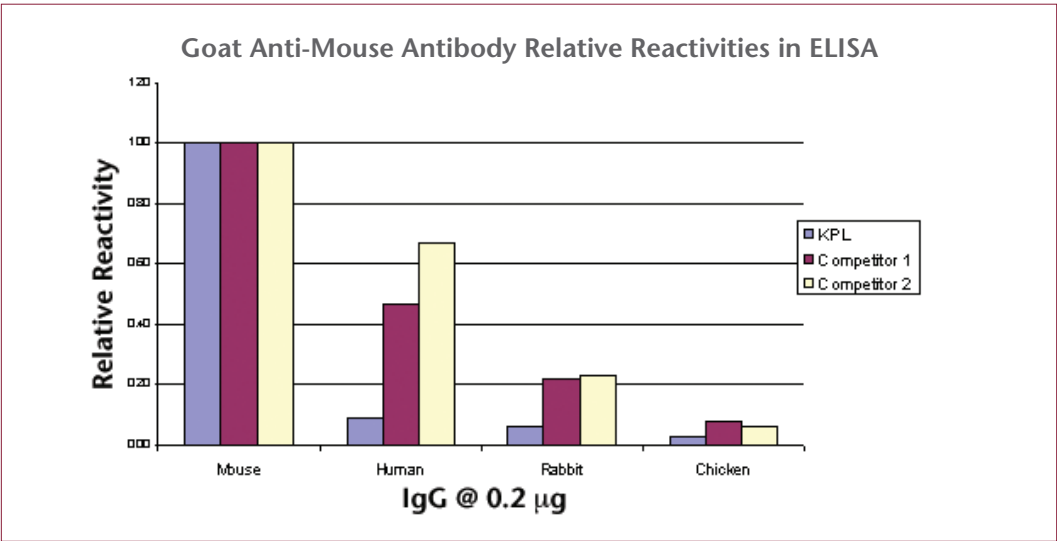
KPL's secondary antibodies improve immunoassay sensitivity and can reduce the amount of precious samples and primary antibodies used. As seen in the figure below, KPL conjugates are highly sensitive.



Western blot study comparing KPL's peroxidase-labeled Goat Anti-Mouse IgG (H+L) antibody with those of leading competitors under identical assay conditions. KPL's antibody demonstrates stronger signal and higher signal-to-noise.

Excellent Specificity

Antibody specificity is critical for immunoassays requiring minimal cross-reactivity and low background. Each KPL antibody has exacting requirements for specificity (see figure below). Both positive and negative affinity adsorptions are done to select antibodies that react strongly with an immunoglobulin target and to remove those antibodies that cross-react with other immunoglobulin or protein.



KPL's Goat Anti-Mouse IgG (H+L) antibody demonstrates superior specificity when compared with the antibodies of two leading competitors.

Lot-to-Lot Consistency

Have you ever spent time optimizing an assay only to find you needed to start over when you bought the next lot of antibody? KPL understands this frustration and reduces lot-to-lot variability using an ISO 9001:2008 quality management systems that ensures consistency lot after lot after lot.

## Our Offerings

KPL's large selection of secondary antibodies consists of class-specific antibodies and combinations of antibodies for simultaneous measurement of several heavy chain classes. The information provided below is intended as a guide in choosing the appropriate antibody class for your assay.

Ig CLASS	CATALOG NO.		ANTIBODY DESCRIPTION
	ANTI-HUMAN	ANTI-MOUSE	
<b>IgA (<math>\alpha</math>)</b>	01-10-01	01-18-01	Specific for IgA. Cross-reactivity to other heavy chain classes (IgG, IgM, IgE) has been removed through affinity chromatography.
<b>IgE (<math>\epsilon</math>)</b>	01-10-04	--	Specific for IgE. Cross-reactivity to other heavy chain classes (IgG, IgM, IgA) has been removed through affinity chromatography.
<b>IgG (<math>\gamma</math>)</b>	01-10-02	01-18-02	Specific for IgG. Cross-reactivity to other heavy chain classes (IgA, IgM, IgE) has been removed through affinity chromatography.
<b>IgM (<math>\mu</math>)</b>	01-10-03	01-18-03	Specific for IgM. Cross-reactivity to other heavy chain classes (IgG, IgA, IgE) has been removed through affinity chromatography.
<b>IgG (H+L)</b>	01-10-06	01-18-06	Detects IgG heavy and light chains and may cross-react with other Ig classes that have light chains in common with IgG. Good choice when broad immunoglobulin reactivity is desired.
<b>IgG+IgM (H+L), HSA</b>	--	01-18-09	Recognizes both IgG and IgM. Even broader reactivity than IgG (H+L).
<b>IgA+IgG+IgM (H+L)</b>	01-10-07	01-18-07	Broadest immunoglobulin detection. Detects IgA, IgG, and IgM.
<b>IgG (Fc)</b>	01-10-20	--	Detects the Fc region of IgG.
<b>F(ab')<sub>2</sub> Fragments</b>	201-1006	201-1806	Pepsin digestion of whole IgG antibodies removes Fc region. Used in applications where non-specific binding via Fc receptors may be an issue.



Please visit [www.kpl.com/antibodies](http://www.kpl.com/antibodies) for a complete listing of polyclonal secondary antibodies, including anti-species immunoglobulin antibodies to bovine, cat, chicken, deer, dog, duck, ferret, goat, guinea pig, hamster, horse, monkey, *Peromyscus leucopus*, rat, rabbit, swine, and turkey.

## Choosing the Right Conjugate

KPL's antibodies are conjugated with a variety of labels for use in a range of detection techniques. The table below lists our labels, features and benefits, and applications.

LABEL	EX/EM*	MAIN FEATURE/BENEFIT	APPLICATIONS
HRP	N/A	High sensitivity and large selection of appropriate substrates	ELISA, Western blotting, IHC
AP	N/A	Longevity of signal, many substrates available	ELISA, Western blotting, IHC
ReserveAP™	N/A	Greater sensitivity than traditional AP conjugates.	ELISA, Western blotting, IHC
Biotin	N/A	Low background, allows for enhanced sensitivity when paired with labeled streptavidin	ELISA, Western blotting, IHC
Gold	N/A	Direct detection without substrate	Lateral flow, Western blotting, IHC
DyLight 405	400/420	Blue; high sensitivity, low background, enhanced photostability	These labels may be used in the following applications: IHC, flow cytometry, Western blotting, microarrays, and Fluorophore-linked immunosorbent assays (FLISA)
DyLight 488	493/518	Green; high sensitivity, low background, enhanced photostability	
FITC	495/528	Green; easily visualized	
R-PE	546/575	Red; brighter and offers greater photostability than traditional fluorophores	
Cy3	550/570	Yellow; brighter and offers greater photostability than traditional fluorophores	
DyLight 549	550/568	Yellow; high sensitivity, low background, enhanced photostability	
TRITC	555/580	Red; good for multiplexing, easily visualized	
DyLight 594	593/618	Orange; high sensitivity, low background, enhanced photostability	
DyLight 633	638/658	Red; high sensitivity, low background, enhanced photostability	
DyLight 649	646/674	Red; high sensitivity, low background, enhanced photostability	
Cy5	650/670	Red; brighter and offer greater photostability than traditional fluorophores	
DyLight 680	682/712	Near Infrared; high sensitivity, low background, enhanced photostability	
DyLight 800	777/794	Infrared; high sensitivity, low background, enhanced photostability	

\*Excitation and emission spectra in nanometers.



### Our Most Popular Antibodies

The table below provides an overview of our most popular secondary antibodies and streptavidin conjugates. They provide sensitive detection in immunoassays such as ELISA, Western blotting, and immunohistochemistry.

LABEL	ANTI-HUMAN IgG (H+L)	ANTI-MOUSE IgG (H+L)	ANTI-RABBIT IgG (H+L)	ANTI-RAT IgG (H+L)	STREPTAVIDIN
Unlabeled	01-10-06	01-18-06	01-15-06	01-16-06	--
HRP	074-1006	074-1806	04-15-06	14-16-06	474-3000
AP	05-10-06	05-18-06	05-15-06	15-16-06	475-3000
ReserveAP™	0751-1006	0751-1806	0751-1506	--	--
Biotin	16-10-06	176-1806	176-1506	16-16-06	57-40-06
Gold	57-10-06	57-18-06	57-15-06	--	57-30-06
FITC	02-10-06	172-1806	172-1506	02-16-12	072-30-00
TRITC	--	03-18-06	03-15-06	03-16-06	073-30-00
Cy3	072-01-10-06	072-01-18-06	072-01-15-06	072-01-16-06	072-01-30-00
Cy5	072-02-10-06	072-02-18-06	072-02-15-06	072-02-16-06	072-02-30-00
RPE	--	0718-18-061	0718-15-061	0718-16-061	--
DyLight 405	072-08-10-06	072-08-18-06	072-08-15-06	072-08-16-06	072-08-30-00
DyLight 488	072-03-10-06	072-03-18-06	072-03-15-06	072-03-16-06	072-03-30-00
DyLight 549	072-04-10-06	072-04-18-06	072-04-15-06	072-04-16-06	072-40-30-00
DyLight 594	072-09-10-06	072-09-18-06	072-09-15-06	072-09-16-06	072-09-30-00
DyLight 633	072-10-10-06	072-10-18-06	072-10-15-06	072-10-16-06	072-10-30-00
DyLight 649	072-05-10-06	072-05-18-06	072-05-15-06	072-05-16-06	072-05-30-00
DyLight 680	072-06-10-06	072-06-18-06	072-06-15-06	072-06-16-06	072-06-30-00
DyLight 800	072-07-10-06	072-07-18-06	072-07-15-06	072-07-16-06	072-07-30-00



See More with KPL BacTrace® Products

# Anti-Bacteria Primary *Antibodies and Controls*

- ➔ Polyclonal antibodies to **food-borne pathogens** enables sensitive detection in a variety of immunoassays.
- ➔ Polyclonal antibodies to **infectious disease agents** facilitate sensitive detection of select bacteria.
- ➔ **Positive Controls** are heat-killed bacteria cells for use in immunoassays.

# Anti-Bacteria Primary Antibodies and Controls

When immunoassay methods for the detection of bacteria are integrated into conventional microbiological procedures, they can greatly reduce time spent on identification and characterization. This is particularly important when there is an outbreak of a food-borne pathogen or infectious disease agent.

KPL leads the food-borne pathogen detection market by enabling immunodetection, basic research, and diagnostic test development through an offering of highly specific polyclonal antibodies to detect *Salmonella*, *Campylobacter*, *Listeria*, and Shiga toxin-producing *E. coli* (STEC).

We also support the human infectious disease research and detection markets by offering a growing range of primary antibodies to detect *Helicobacter pylori*, *Yersinia pestis*, and *Borrelia burgdorferi*.

## Our Offerings

### BacTrace® Antibodies

#### BacTrace Antibodies to Food-borne Pathogens

KPL's line of BacTrace polyclonal antibodies are directed to a series of food-borne pathogens that cause significant disease and economic cost worldwide. KPL offers antibodies to *E. coli*, *Salmonella* CSA-1, *Listeria*, *Campylobacter*, *Vibrio cholera*, and *Shigella*. They enable rapid, sensitive detection in a variety of assay formats.

#### Shiga Toxin-producing *E. coli* Antibodies

Shiga toxin-producing *E. coli* (STEC) are responsible for numerous outbreaks traced to the consumption of contaminated foods and beverages. Although *E. coli* O157:H7 is responsible for the majority of human illnesses, the USDA recently expanded its definition of *E. coli* adulterants to include STEC O26, O111, O121, O45, O145, and O103, all of which cause hemorrhagic colitis and post-diarrheal hemolytic uremic syndrome, with potentially deadly outcomes. BacTrace anti-bacteria polyclonal antibodies provide a powerful set of tools to identify STEC food pathogens with minimal cross-reactivity.

#### BacTrace Antibodies to Infectious Disease Agents

KPL is expanding its line of BacTrace polyclonal antibodies to select bacteria that cause significant infectious disease in humans. Included in this line are antibodies to *Borrelia burgdorferi* (Lyme disease), *Helicobacter pylori* (causative agent of stomach ulcers), *Legionella* (Legionellosis), and *Yersinia pestis* (plague). These antibodies are powerful tools for research and development of detection assays.

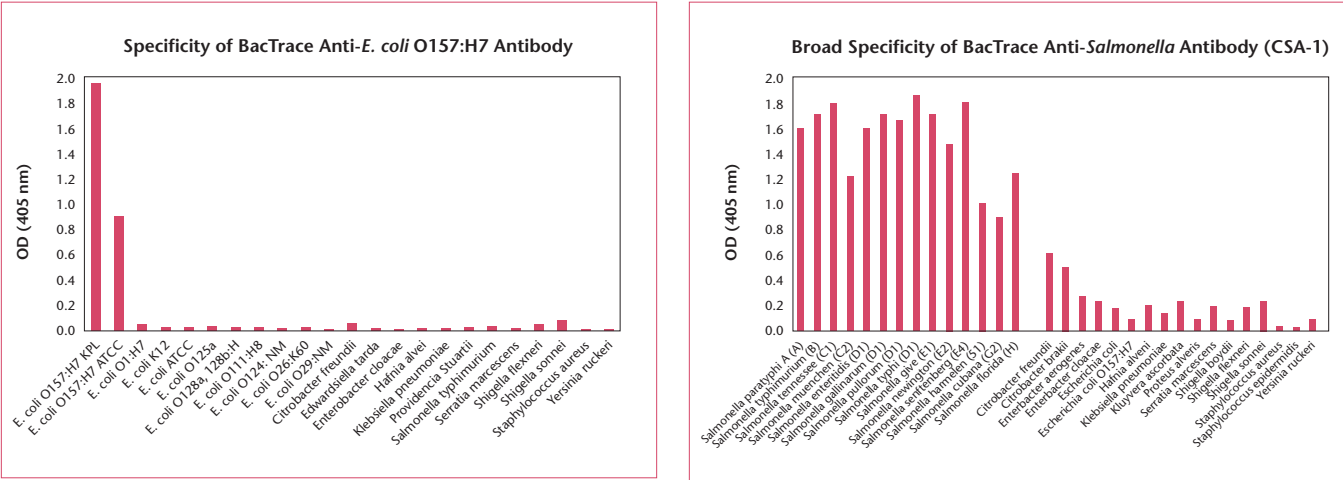
#### BacTrace Bacteria Positive Controls

BacTrace bacteria positive controls are made from heat-killed bacteria cells and are recommended for use as positive controls in immunoassays.



Broad Spectrum and Serotype-Specific Detection

KPL's unique purification protocols enable the production of both highly specific (Figure 1) and broadly reactive (Figure 2) antibodies.



**Figures 1 (left) and 2 (right):**  
Colorimetric ELISA demonstrates highly specific reactivity of BacTrace Anti-E.coli O157:H7 antibody for the O157:H7 serotype with minimal reactivity to other potentially cross-reacting serotypes. BacTrace Anti-Salmonella antibody demonstrates broad reactivity to Salmonella serotypes including those significant in human disease.

Support Reagents

See pages 4, 9, 22 for ELISA, Western blot, and secondary antibodies, respectively.

## BacTrace Anti-Bacteria Antibodies Selection Guide

	CATALOG NO. AND SIZE				
	UNLABELED	HRP	BIOTIN	DYLIGHT 488	POSITIVE CONTROLS
<b>Antibodies to Food Pathogens</b>					
<b>Goat Anti-Campylobacter species</b>	01-92-93, 1.0 mg	04-92-93, 0.1 mg			50-92-93, 1 vial
<b>Goat Anti-E.coli O103</b>	01-95-93, 1.0 mg 011-95-93, 0.1 mg	04-95-93, 0.1 mg	16-95-93, 0.5 mg	072-95-93, 0.5 mg	50-95-93, 1 vial
<b>Goat Anti-E.coli O111</b>	01-95-91, 1.0 mg 011-95-91, 0.1 mg	04-95-91, 0.1 mg	16-95-91, 0.5 mg	072-03-95-91, 0.5 mg	50-95-91, 1 vial
<b>Goat Anti-E.coli O121</b>	01-95-95, 1.0 mg 011-95-95, 0.1 mg	04-95-95, 0.1 mg	16-95-95, 0.5 mg	072-03-95-95, 0.5 mg	50-95-95, 1 vial
<b>Goat Anti-E.coli O145</b>	01-95-94, 1.0 mg 011-95-94, 0.1 mg	04-95-94, 0.1 mg	16-95-94, 0.5 mg	072-03-95-94, 0.5 mg	50-95-94, 1 vial
<b>Goat Anti-E.coli O157:H7</b>	01-95-90, 1.0 mg	04-95-90, 0.1 mg	16-95-90, 0.5 mg		50-95-90, 1 vial
<b>Goat Anti-E.coli O157:H7 Molecular Grade</b>	01-95-90-MG, 1.0 mg				
<b>Goat Anti-E.coli O26</b>	01-95-92, 1.0 mg 011-95-92, 0.1 mg	04-95-92, 0.1 mg	16-95-92, 0.5 mg	072-03-95-92, 0.5 mg	50-95-92, 1 vial
<b>Goat Anti-E.coli O45</b>	01-95-96, 1.0 mg 011-95-96, 0.1 mg	04-95-96, 0.1 mg	16-95-96, 0.5 mg	072-03-95-96, 0.5 mg	50-95-96, 1 vial
<b>Goat Anti-Listeria species</b>	01-90-90, 1.0 mg	04-90-90, 0.1 mg	16-90-90, 0.5 mg		50-90-90, 1 vial
<b>Goat Anti-Listeria species High Sensitivity</b>	01-90-95, 1.0 mg				
<b>Goat Anti-Salmonella Common Structural Antigen (CSA-1)</b>	01-91-99, 1.0 mg	04-91-99, 0.1 mg	16-91-99, 0.5 mg		50-74-01, 1 vial
<b>Goat Anti-Salmonella Common Structural Antigen (CSA-1) Molecular Grade</b>	01-91-99-MG, 1.0 mg				
<b>Rabbit Anti-Shigella species</b>	01-90-01, 1.0 mg	04-90-01, 0.1 mg	16-90-01, 0.5 mg	072-03-90-01, 0.5 mg	50-90-01, 1 vial
<b>Rabbit Anti-Vibrio cholera</b>	01-90-50, 0.5 mg				
<b>Rabbit Anti-Vibrio species</b>	01-90-02, 1.0 mg	04-90-02, 0.1 mg	16-90-02, 0.5 mg	072-03-90-02, 0.5 mg	50-90-02, 1 vial
<b>Antibodies to Infectious Disease Agents</b>					
<b>Goat Anti-Borrelia burgdorferi</b>	01-97-91, 1.0 mg	04-97-91, 0.1 mg			50-97-91, 1 vial
<b>Goat Anti-Borrelia species</b>	01-97-92, 1.0 mg	04-97-92, 0.1 mg			
<b>Goat Anti-Helicobacter pylori</b>	01-93-94, 1.0 mg	04-93-94, 0.1 mg			50-93-94, 1 vial
<b>Rabbit Anti-Legionella species</b>	01-90-03, 1.0 mg	04-90-03, 0.1 mg	16-90-03, 0.5 mg	072-03-90-03, 0.5 mg	50-90-03, 1 vial
<b>Rabbit Anti-Yersinia species</b>	01-90-04, 1.0 mg	04-90-04, 0.1 mg	16-90-04, 0.5 mg	072-03-90-04, 0.5 mg	50-90-04, 1 vial
<b>Other Anti-Bacteria Antibodies</b>					
<b>Goat Anti-Renibacterium salmoninarum</b>	01-96-91, 1.0 mg	04-96-91, 0.1 mg			50-96-91, 1 vial



Please visit [www.kpl.com/BacTrace](http://www.kpl.com/BacTrace) to see our fluorescein- and phosphatase-labeled BacTrace antibodies, detailed product line information, latest pricing, and easy ordering information.



See More with KPL SureLink™ Products

A photograph of a tiger cub in a forest, partially obscured by green leaves and brown leaves on the ground. The cub is looking towards the camera.

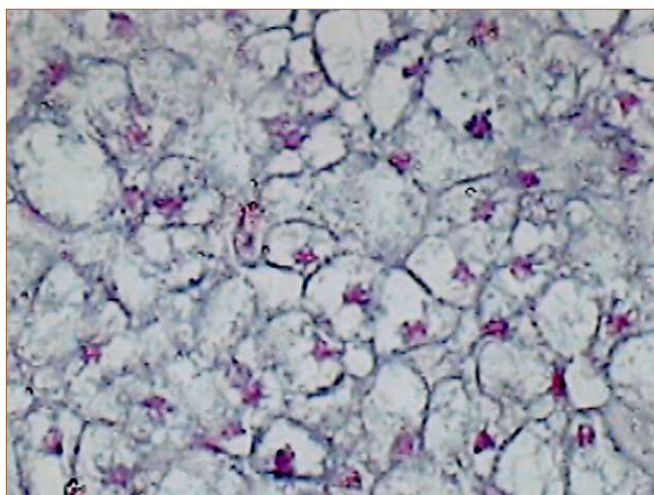
## Protein Labeling *Kits and Reagents*

- **SureLINK HRP Conjugation Kits and Reagents** contain pre-activated HRP supplied in reaction-size vials capable of conjugating a variety of proteins.
- **SureLINK AP Conjugation Kits** contain modified AP in reaction-size vials capable of conjugating a variety of proteins.
- **SureLINK Fluorescein Labeling Kits** label proteins with the fluorophores 5-FITC or 5-FAM-X.
- **SureLINK Chromophoric Biotin Labeling Kits** provide an easy method for biotinylating antibodies and other proteins and for accurately measuring biotin incorporation.



# Protein Labeling Kits and Reagents

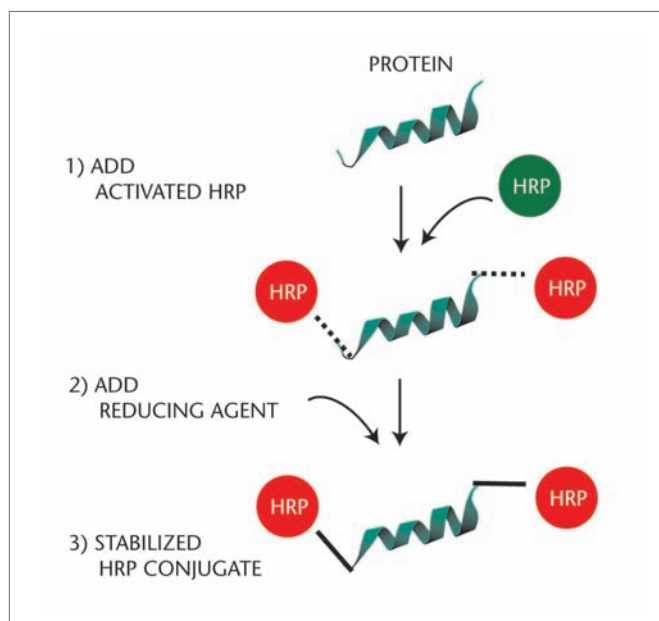
KPL's SureLINK™ protein labeling products provide a convenient and efficient means for labeling proteins and antibodies with enzymes, biotin, or fluorescein through primary amine groups. KPL's labeling kits contain all the reagents required for easy preparation of highly stable, conjugated antibodies and proteins ideal for use in immunodetection assays.



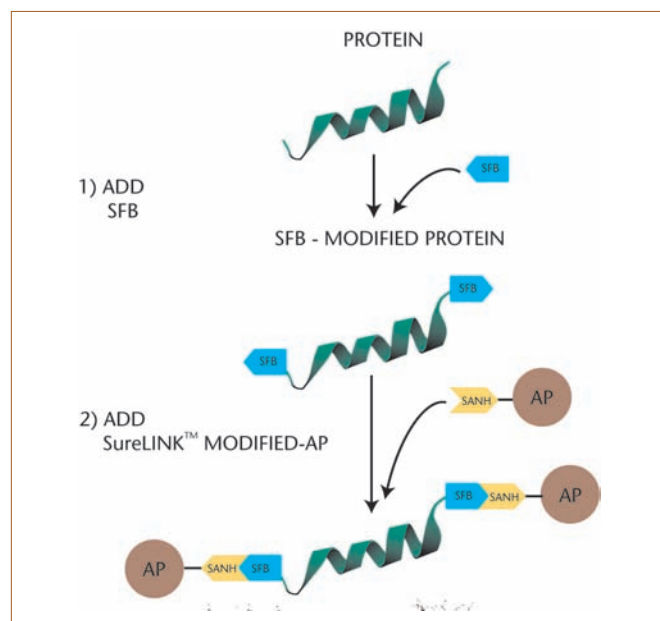
*Detection of epithelial membrane antigen in human liver tissue by immunohistochemistry using SureLink™ Chromophoric Biotin conjugates. Slides were stained/counterstained with TrueBlue/Contrast Red, resulting in a blue/purple and pink stain of the antigen and nuclei, respectively.*

See how easy it is to produce consistent and reproducible conjugates in just a few steps when labeling antibodies with SureLINK HRP or AP Conjugation Kits (see figures below).

## SureLINK HRP Protocol



## SureLINK AP Protocol



## Our Offerings

### Choosing a SureLINK Kit for Labeling Your Protein or Antibody

Reagents are packaged in vials sized for a particular number of reactions with all the essential components for conjugation and purification provided and an easy step-by-step guide. Final conjugates have broad application in a variety of immunoassays, including ELISA, Western blotting, protein microarrays, and immunohistochemistry. When selecting a kit, consider all the criteria below.

SURELINK KITS	CATALOG NO.	KIT CONTENTS	# REACTIONS	LABELING CHEMISTRY	TIME TO RESULTS
SureLINK HRP Conjugation Kits	84-00-01 84-00-02 84-00-03	<ul style="list-style-type: none"> <li>Activated HRP</li> <li>HRP Conjugation Buffer</li> <li>Reducing Reagent</li> <li>HRP Storage Buffer</li> </ul>	6 x 0.1 mg rxn 6 x 1 mg rxn 2 x 0.1 mg rxn	Two-step protocol based on periodate chemistry. HRP is pre-activated and supplied in reaction size vials.	1.5 hours <20 minutes hands-on time
SureLINK AP Conjugation Kits	85-00-01 85-00-02	<ul style="list-style-type: none"> <li>Modified AP</li> <li>AP Modification Buffer</li> <li>SFB Linker</li> <li>AP Conjugation Buffer</li> <li>AP Storage Buffer</li> </ul>	3 x 0.5 mg rxn 3 x 0.1 mg rxn	Two-step protocol based on novel conjugation chemistry superior to maleimide/-thiol, glutaraldehyde and avidin/biotin.	3 hours <20 minutes hands-on time
SureLINK Fluorescein (FITC) Labeling Kit	82-00-01	<ul style="list-style-type: none"> <li>FITC</li> <li>5 Carbonate Bi-carbonate Buffer Capsules</li> <li>Anhydrous DMF</li> <li>Spin-Pure Filters</li> <li>Reaction Tubes</li> </ul>	5 x 0.3 mg rxn	Labels proteins with the fluorophore 5-FITC	1.5 - 2 hours <30 minutes hands-on time
SureLINK Fluorescein-X (FAM-X) Labeling Kit	82-00-02	<ul style="list-style-type: none"> <li>FAM-X</li> <li>Borate Buffer</li> <li>Anhydrous DMF</li> <li>Spin-Pure Filters</li> <li>Reaction Tubes</li> </ul>	5 x 0.3 mg rxn	Labels proteins with 5-FAM-X (FAM fluorophore with succinimidyl ester and a seven atom aminohexanoyl spacer, known as 'X'), reduces quenching.	1.5 - 2 hours <30 minutes hands-on time
SureLINK Chromophoric Biotin Labeling Kit	86-00-01	<ul style="list-style-type: none"> <li>Activated Biotin</li> <li>Reconstitution Solvent</li> <li>Modification Buffer</li> <li>Reconstitution Solvent</li> <li>Spin filters</li> </ul>	5 x 1 mg biotin rxn	Long spacer arm reduces steric hindrance. Easy biotinylation method. Accurately measures biotin incorporation using simple spectrophotometric analysis at 354 nm.	3 hours <30 minutes hands-on time



Select reagents are available separately and can be used with your protocols and reagents. Please visit [www.kpl.com/proteinlabeling](http://www.kpl.com/proteinlabeling) for our complete line of products, latest pricing, and ordering information.



See More with KPL HisDetector™ Products

# Recombinant Protein *Detection and Analysis*

- ➔ **HisDetector Nickel Conjugates** provide direct detection of recombinant proteins.
- ➔ **HisDetector Western Blotting Kits** are available using either HRP or AP nickel conjugates and your choice of chemiluminescent or colorimetric detection.

# Recombinant Protein Detection and Analysis

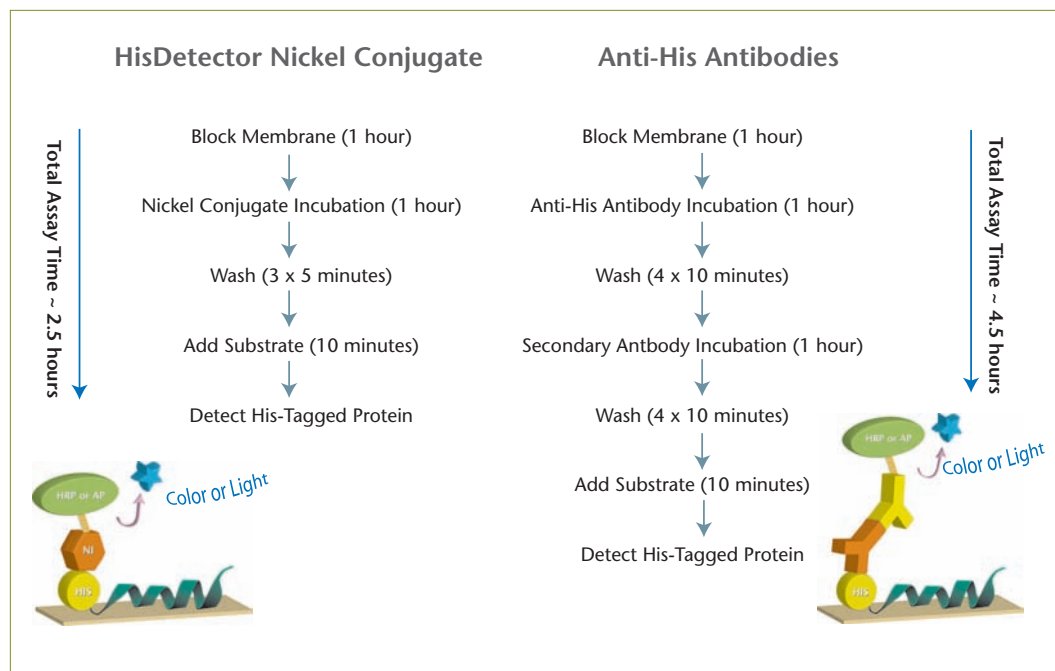
HisDetector™ Nickel Conjugates and Western blot kits provide a fast and reliable method for directly detecting His-tagged recombinant proteins. Detection is based on nickel-nitrilotriacetic acid (NTA), which binds with high affinity to histidine residues. They enable simple, direct detection of His-tagged proteins.

Using unique technology, KPL has coupled nickel-NTA to HRP and AP. These nickel conjugates form the basis of the HisDetector products. They are offered with three Western blot kits to provide high sensitivity without background.

## Our Offerings

### HisDetector Nickel Conjugates

HisDetector Nickel Conjugates provide direct detection of recombinant proteins without the use of primary and secondary antibodies.



*Comparison of direct detection using nickel conjugates with indirect detection using anti-His in a Western blot.*



## HisDetector Nickel Conjugates Comparison and Selection Guide

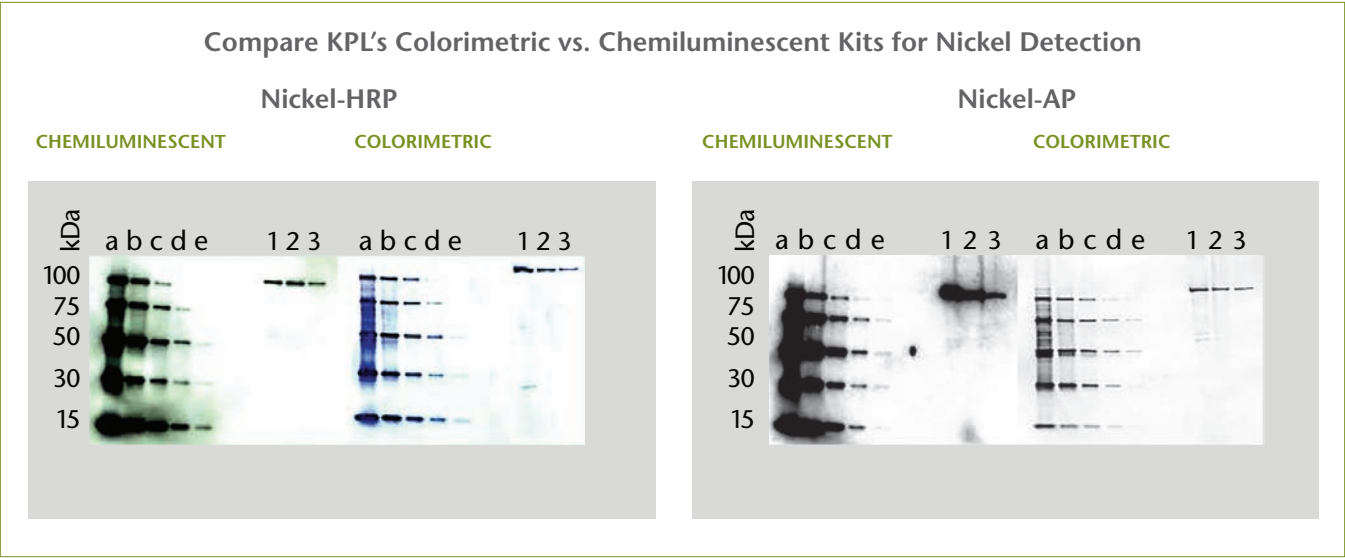
DESCRIPTION	CATALOG NO.	ENZYME	CONTENTS	SENSITIVITY	SPECIFICITY	BACKGROUND	APPLICATIONS
HisDetector Nickel-HRP Conjugate	24-01-01	HRP	Nickel-HRP (0.1 mL)	Picogram	High	Low	ELISA, Western blot, immunohistology assays
HisDetector Nickel-AP Conjugate	25-01-01	AP	Nickel-AP (1.0 mL) and Detector® Block Solution (120 mL)	Picogram	High	Low	ELISA, Western blot, immunohistology assays

## HisDetector Western Blot Kits

HisDetector Western Blot Kits provide a choice of chemiluminescent or colorimetric detection using either Nickel-HRP or Nickel-AP conjugates. They can detect picogram levels of His-tagged proteins in cell extracts with superior sensitivity and lower background than antibody-based methods, ensuring detection even at low expression levels. These kits offer the most rapid means available for His-tagged protein detection in a Western blot.

## HisDetector Western Blot Kits Comparison and Selection Guide

DESCRIPTION	CATALOG NO.	ENZYME	KIT CONTENTS	SENSITIVITY	SPECIFICITY	BACKGROUND
HisDetector Western Blot Kit, 40 blots	24-00-01	HRP Colorimetric	Nickel-HRP, blocking solution, wash solution concentrate and substrate	Picogram	High	Low
HisDetector Western Blot Kit, 40 blots	24-00-02	HRP Chemiluminescent	Nickel-HRP, blocking solution, wash solution concentrate and substrate	Picogram	High	Low
HisDetector Western Blot Kit, 40 blots	25-00-01	AP Colorimetric	Nickel-AP, blocking solution, wash solution concentrate and substrate	Picogram	High	Low



*His-tagged protein ladder (lanes a-e) and E. coli crude lysate containing His-tagged  $\beta$ -gal (lanes 1-3) were serially diluted and detected using HisDetector Western Blot Kits.*



Please visit [www.kpl.com/Histag](http://www.kpl.com/Histag) for our complete line of products, latest pricing, and easy ordering information.



See More with KPL Protein Purification Products

# Sample Preparation *and* Protein Purification

- ➔ **Immunoprecipitation Kits** are a versatile set of Protein A or Protein G Agarose reagents and protease inhibitors for a variety of applications.
- ➔ **Protein A and Protein G Purification Kits** offer superior performance in both immunoprecipitation and antibody purification.
- ➔ **Protein A and Protein G Agarose** are used to isolate and purify mammalian immunoglobulins.

# Sample Preparation and Protein Purification

KPL offers a versatile set of reagents and kits for protein purification and immunoprecipitation for downstream protein analysis and detection.

Immunoprecipitation is a technique used to selectively purify an antigen of interest from a complex mixture (e.g., supernatant) in advance of an immunodetection process like ELISA or Western blot. Purification is accomplished through the use of a specific antibody and a solid phase matrix attached to Protein A or G to bring the antigen-antibody complex out of solution. This technique is often used to study the presence or absence of an antigen, the quantity of an antigen at a given time point, relative molecular weights of proteins, protein-protein interactions, post-translational modifications, or the rate of synthesis or degradation of antigens.

Immunopurification provides a means of nonspecifically separating antibodies from a complex mixture such as ascites or cell culture fluid.

## Binding Characteristics of Protein A and Protein G

The table below shows the relative binding affinity of Protein A and Protein G for various antibody classes and subclasses of monoclonal and polyclonal antibodies from mammalian species. Use it as a guide in choosing from our offering of Protein A or Protein G on an agarose bead, or kits for purification and immunoprecipitation.

Monoclonal			Polyclonal		
ANTIBODY	BINDING		ANTIBODY	BINDING	
	Protein A	Protein G		Protein A	Protein G
Human IgG <sub>1</sub>	++++	++++	Rabbit	++++	+++
Human IgG <sub>2</sub>	++++	++++	Cow	++	++++
Human IgG <sub>3</sub>	---	++++	Horse	++	++++
Human IgG <sub>4</sub>	++++	++++	Goat	-	++
Mouse IgG <sub>1</sub>	+	++++	Guinea Pig	++++	++
Mouse IgG <sub>2a</sub>	++++	++++	Sheep	+/-	++
Mouse IgG <sub>2b</sub>	+++	+++	Pig	+++	+++
Mouse IgG <sub>3</sub>	++	+++	Rat	+/-	++
Rat IgG <sub>1</sub>	---	+	Mouse	++	++
Rat IgG <sub>2a</sub>	---	++++	Chicken	---	+
Rat IgG <sub>2b</sub>	---	++	Human IgG	++++	++++
Rat IgG <sub>2c</sub>	+	++	Human IgM	---	+
			Human IgD	---	+
			Human IgA	---	+

+ strong binding      - weak or no binding



## Our Offerings

KPL provides high quality Protein A and Protein G Agarose as stand-alone reagents for the isolation and purification of IgG molecules. These reagents are also the foundation of fully optimized kits for immunoprecipitation and antibody purification and are selected for superior performance in these applications.

### Protein A and Protein G Agarose

Protein A and Protein G Agarose are suitable for incorporation into a variety of purification and immunoprecipitation applications for isolation of antigen-antibody complexes. Protein A Agarose consists of native Protein A immobilized onto cross-linked agarose beads. Protein G Agarose consists of recombinant Protein G, produced in *E. coli* and covalently immobilized onto cross-linked agarose beads.

	CATALOG NO.	CONTENTS	BINDING CAPACITY	STRENGTHS	LIMITATIONS
<b>Protein A Agarose</b>	223-50-01	Protein A ligand immobilized onto 4% cross-linked agarose beads	35 mg/mL human IgG per mL resin	Heat stable and stable in 4M Urea, 4M guanidine thiocyanate. Binds specifically to Fc region without disturbing antigen binding	Affinity for fewer IgG's than Protein G
<b>Protein G Agarose</b>	223-51-01	Recombinant Protein G immobilized onto 4% cross-linked agarose beads	20 mg/mL human IgG per mL resin	Recombinant Protein G optimized for non-specific binding. Can bind Fc region and Fab fragments. Affinity for more IgG's than Protein A	Has a lower binding capacity per mL than Protein A

### Immunoprecipitation Kits

KPL's immunoprecipitation kits provide a versatile set of reagents, protease inhibitors, and Protein A or Protein G Agarose that can be used for a variety of applications. Sufficient reagents are provided to lyse cells, solubilize, and capture antigens from 20 x 1 mL cell aliquots.

	CATALOG NO.	COMPONENTS	SIZE	APPLICATION
<b>Protein A Agarose Immunoprecipitation Kit</b>	553-50-01	Buffers, washes, protease inhibitors, and Protein A agarose reagents	20 reactions	To purify functional protein from complex mixtures for subsequent applications
<b>Protein G Agarose Immunoprecipitation Kit</b>	553-51-01	Buffers, washes, protease inhibitors, and Protein G agarose reagents	20 reactions	To purify functional protein from complex mixtures for subsequent applications

Protein A and Protein G Purification Kits

KPL's protein purification kits contain all of the reagents needed to purify antibodies, as well as instructions for use. High quality Protein A and Protein G Agarose are the foundation of KPL's purification systems. Each resin has been selected for its superior performance in antibody purification applications. The buffer system is optimized to permit high recovery of pure antibody from crude serum samples.

	CATALOG NO.	COMPONENTS	SIZE	APPLICATION
Protein A Agarose Purification Kit	553-50-00	Buffers, washes, purification column, and Protein A agarose reagents	5 mL	For purification of Igs from mammalian species; resin may be used multiple times
Protein G Agarose Purification Kit	553-51-00	Buffers, washes, purification column, and Protein G agarose reagents	10 mL	For purification of Igs from mammalian species; resin may be used multiple times



Please visit [www.kpl.com/proteinpurification](http://www.kpl.com/proteinpurification) for our complete line of products, latest pricing, and easy ordering information.



See More with KPL Support Reagent Products

# Immunoassay Support Reagents

- ➔ Protein and non-protein **blocking solutions** reduce non-specific binding and background.
- ➔ **Washing solutions** compatible with most assay systems are effective in minimizing non-specific binding to the solid phase.
- ➔ **Buffers** are offered as convenient liquid concentrates.
- ➔ Easy-to-use **stop solutions** halt enzyme substrates at a specific point in color development for consistent results.
- ➔ **Conjugate stabilizers** ensure consistent activity over time.

# Immunoassay Support Reagents

All immunoassays require support reagents. KPL's assay reagents include blocking solutions, wash solutions, buffers, and conjugate stabilizers. They are formulated to ensure accurate, reproducible results. The reagents are optimized for use in microwell ELISA, Western blotting, and immunohistochemical applications.

## Blocking Solutions

Before using antibodies to detect proteins on a membrane, microwell plate, or tissue sample, the remaining binding surface must be blocked to prevent non-specific binding of the antibodies. Any protein that does not have binding affinity for the target in the assay can be used for blocking a surface. However, certain proteins perform better than others as they bind to the immobilization surface more consistently, do not obscure the epitope of interest, and help reduce background, thereby improving the signal-to-noise ratio. To find the right reagent for your assay, choose from our offering of protein and non-protein blockers.

	CATALOG NO.*	BLOCKING INGREDIENT	DESCRIPTION	RECOMMENDED APPLICATIONS
<b>Specialty Blockers</b>				
<b>SignalLOCK™</b>	50-58-00	Proprietary non-protein compound	Premium non-protein, non-animal origin blocker provides excellent blocking without obscuring epitopes of interest. Extremely stable. Supplied as 125 mL concentrate.	WB†
<b>5X Detector Block</b>	71-83-00	Fish gelatin, sodium caseinate	Fish gelatin blocker is effective at blocking background without obscuring epitopes of interest. Supplied as 240 mL concentrate with a powder component.	WB, Nucleic Acid Detection, ELISA
<b>Standard Blockers</b>				
<b>10% BSA Diluent Blocking Solution</b>	50-61-00	10% Bovine serum albumin	Pure protein provides less cross-reactivity than serum and milk. Easy-to-use liquid. Good, all-purpose blocker. Supplied as 200 mL concentrate.	ELISA, WB, IHC
<b>Milk Diluent Blocking Solution</b>	50-82-01	2% Nonfat milk	Excellent milk blocker is virtually clear. Easy-to-use liquid. Supplied as 200 mL concentrate.	ELISA, WB
<b>Species-specific Blockers</b>				
<b>Normal Goat Serum</b>	71-00-27	Goat serum proteins	Alternative to BSA or milk blockers. Use when primary antibody produced in goat. Supplied as 50 mL concentrate.	IHC†, ELISA, WB
<b>Normal Rabbit Serum</b>	71-00-28	Rabbit serum proteins	Alternative to BSA or milk blockers. Use when primary antibody produced in rabbit. Supplied 50 mL concentrate.	IHC†, ELISA, WB
<b>Normal Mouse Serum</b>	71-18-01	Mouse serum proteins	Alternative to BSA or milk blockers. Use when primary antibody produced in mouse. Supplied as 10 mL concentrate.	IHC†, ELISA, WB
<b>Endogenous Enzyme Blockers</b>				
<b>Universal Block</b>	71-00-61	Proprietary reagent	Blocks endogenous HRP and AP. Especially useful in double-labeling assays. Supplied as 100 mL solution ready-to-use.	IHC
<b>Blocking Solution Concentrate</b>	71-00-10	Periodic Acid	Blocks endogenous HRP. Supplied as a 10X concentrate.	IHC

\* Smallest catalog size is listed. Other sizes are available.

† Primary application



## Wash Solutions

Wash solutions are critical for reducing nonspecific binding to a solid phase such as microwell, membrane, or tissue.

	CATALOG NO.*	WASH INGREDIENT	DESCRIPTION	RECOMMENDED APPLICATIONS
<b>Specialty Wash Solutions</b>				
<b>Wash Solution Concentrate</b>	50-63-00	Unique formulation of imidazole-buffered saline and Tween 20.	Supplied as 800 mL concentrate	General immunoassay wash buffer
<b>Phosphatase Wash Solution</b>	50-63-15	PBS-based	Supplied as 600 mL concentrate	General immunoassay wash buffer for use in detection of alkaline phosphatase conjugates on membranes.
<b>Biotin Wash Solution</b>	50-63-06	Patented proprietary formulation	Reduces background due to nonspecific binding of enzyme-labeled streptavidin to nylon membranes and tissues. Supplied as 200 mL concentrate.	Use in biotin-streptavidin applications such as nucleic acid and Western blotting, colony and plaque lifts, and <i>in situ</i> hybridization.
<b>Standard Wash Solutions</b>				
<b>TBST with 0.5% Tween 20</b>	51-18-01	1X formulation: 50 mM Tris, 150 mM NaCl, 0.05% Tween 20, pH 7.6	Supplied as a 10X concentrate	General immunoassay wash buffer
<b>TBST with 10% Tween 20</b>	51-19-01	1X formulation: 50 mM Tris, 150 mM NaCl, 1.0% Tween 20, pH 7.6	Supplied as a 10X concentrate	General immunoassay wash buffer
<b>PBST</b>	51-14-01	1X formulation: 10 mM Phosphate, 150 mM NaCl, 0.05% Tween 20, pH 7.4	Supplied as a 10X concentrate	General immunoassay wash buffer
<b>Dulbecco's PBST</b>	51-16-01	1X formulation: 8.5 mM sodium phosphate, 1.5 mM potassium phosphate, 137 mM NaCl, 2.7 mM KCl, 0.05% Tween 20, pH 7.4	Supplied as a 10X concentrate	General immunoassay wash buffer

\* Smallest catalog size is listed. Other sizes are available.

## Buffers

KPL's line of general immunoassay buffers eliminates the need for time-consuming reagent preparation. They provide a reliable medium for developing an assay and contribute to consistent results. Our offering includes Dulbecco's PBS, PBST, TBS, Tris-Glycine SDS, and Tris-Glycine Transfer Buffer. All are offered as convenient liquid concentrates. Other benefits include:

- Defined pH range
- Controlled conductivity
- Dilute-and-use format



For a complete listing, go to [www.kpl.com/supportreagents](http://www.kpl.com/supportreagents)

## ELISA Substrate Stop Solutions

KPL manufactures easy-to-use, stable substrate stop solutions. Stopping enzyme substrates at a specific point in color development contributes to consistent ELISA results.

	CATALOG NO.*	STOPPING REAGENT	SUBSTRATES STOPPED	DESCRIPTION
<b>ABTS Peroxidase Stop Solution</b>	50-85-01	5% Sodium dodecyl sulfate (SDS)	ABTS 2-C, ABTS 1-C	Common detergent quickly stops ABTS substrate reactions. Substrate color does not change upon stopping.
<b>TMB Stop Solution</b>	50-85-05	1% HCl	TMB 2-C, SureBlue™, SureBlue Reserve™	Stops TMB through acidification. Stopped TMB turns yellow and is read at 450 nm.
<b>TMB BlueSTOP™</b>	50-85-30	Proprietary	TMB 2-C, SureBlue™, SureBlue Reserve™	Unique stop solution stops TMB without changing TMB's color. Read at 650 nm.
<b>APStop™ Solution</b>	50-89-00	EDTA	pNPP, BluePhos®, FirePhos™	Excellent stop solution for all phosphatase microwell substrates.

\* Smallest catalog size is listed. Other sizes are available.

## Conjugate Stabilizers

KPL conjugate stabilizers prevent irreversible denaturation of enzymes for long-term storage without loss of activity or function.

### APStabilizer

AP Stabilizer is formulated to prevent the breakdown of phosphatase conjugates in diluted form for extended periods of time with minimal effect on conjugate activity.

### HRPStabilizer

HRP Stabilizer is formulated for both the reconstitution and dilution of HRP conjugates.



Looking for other support reagents and accessories? Our full offering of tools to improve your assays can be found at [www.kpl.com/supportreagents](http://www.kpl.com/supportreagents)



**Time Tested, Partner Proven**

# Custom/Bulk *Products and Services*

- ➔ Fourth decade of **leadership**
- ➔ **Bulk and customized packaging** of KPL antibodies, substrates, and immunoassay reagents
- ➔ **Custom antibody purification**
- ➔ **Custom conjugation**
- ➔ **Modification** of existing products



# Custom/Bulk *Products and Services*

## **Trust the Immunodetection Pioneer**

For almost four decades KPL has provided custom and bulk products and services to leading manufacturers of commercially successful kits, including diagnostic, biopharmaceutical, biotech, and food pathogen test manufacturers. Our reagents are integral components in millions of human and animal *in vitro* diagnostic kits, in-process testing protocols, and high-throughput screening applications.

Commercial partners know to rely on KPL's vigorous quality systems and turn to KPL as their preferred vendor and trusted OEM source. KPL is registered and/or approved under the following regulatory agencies:

- ISO 9001:2008
- APHIS (Animal and Plant Health Inspection Service)
- USDA-approved animal facility

## **Superior Immunoassay Products and Services to Meet Your Specifications**

KPL offers a comprehensive portfolio of more than 800 immunoassay products that can power your ELISA, blotting, and immunohistochemistry applications.

- Polyclonal secondary antibodies from goat and rabbit sources, unlabeled or labeled with enzymes, fluorochromes, biotin, or gold
- Colorimetric and chemiluminescent kits and stable liquid substrates for peroxidase and phosphatase detection
- Immunoassay support reagents, including blocks, washes, buffers, and stopping reagents



## *Our Offerings*

### **Bulk and Custom Packaging**

Products are available off the shelf or can be customized to meet your requirements. KPL will accommodate your needs whether you need bulk sizing, or packaged and labeled according to your specifications.

### **Modifications to Existing Products**

If your specifications fall outside those of our standard products, KPL provides a cost-effective approach to achieving custom reagents. Using existing, time-tested products as a starting point, we can make modifications to meet a specific set of parameters.

### **Custom Antibody Purifications**

KPL can purify your polyclonal or monoclonal antibody quickly and efficiently with our proprietary affinity purification technology. Other options available include Protein A, Protein G, and ion exchange purifications.

### **Custom Antibody Conjugations**

KPL is an expert in antibody conjugation and can conjugate your polyclonal or monoclonal with enzymes, fluorochromes, biotin, colloidal gold, and streptavidin to meet your assay needs.

## **Start Your Project Now!**

Our team of scientists has decades of combined experience and can provide dedicated focus and support for your project from start to finish. Let us assist you in selecting technologies and reagents that will enhance your product offerings and shorten your development cycle and time to market. Turnaround time averages 4–6 weeks from start to finished goods, depending on the scope of your project.

Contact us at [oem@kpl.com](mailto:oem@kpl.com) or phone 301.948.7755 option 502 to discuss your needs. We look forward to talking to you.



See [www.kpl.com/oem](http://www.kpl.com/oem) for more information on KPL's products and services.

# Alphabetical Index

ABTS Microwell Peroxidase Stop Solution.....	46	Antibody Labeling Kits .....	34	PhosphaGLO AP.....	8, 14
ABTS Microwell Peroxidase Substrate Systems.....	7	Gold-Labeled .....	26, 27	PhosphaGLO Reserve AP .....	8, 14
Activated Enzymes, AP and HRP .....	34	DyLight Conjugates .....	16, 26	Conjugate Stabilizers.....	47
Alkaline Phosphatase Conjugates .....	22	Secondary Polyclonal, Unlabeled and Labeled... 22, 25		Conjugation Kits and Reagents .....	32
Alkaline Phosphatase Substrates.....	7, 15	APStabilizer.....	47	Colorimetric Substrate .....	21
Anti-Bacteria Antibodies, Conjugates .....	28	APStop Solution .....	46	ELISA Substrates.....	7
<i>Borrelia burgdorferi</i> Antibody.....	31	AP Chemiluminescent Blotting Kit.....	12	Blotting Substrates .....	15
<i>Borrelia burgdorferi</i> Positive Control .....	31	AP Chemiluminescent Substrates .....	8, 14	Counterstains .....	21
<i>Borrelia</i> species Antibody .....	31	AP Nickel Conjugates .....	37	Contrast BLUE.....	21
<i>Campylobacter</i> species Antibody.....	31	AP Protein Labeling Kits .....	34	Contrast GREEN.....	21
<i>Campylobacter jejuni</i> , Positive Control .....	31	Assay Support Reagents.....	43	Contrast RED.....	21
<i>E. coli</i> O26 Antibody.....	31	BCIP/NBT Substrate .....	15	DAPI.....	21
<i>E. coli</i> O26, Positive Control .....	31	Biotin Conjugates.....	19, 27	Orcein .....	21
<i>E. coli</i> O45 Antibody.....	31	Biotin Wash Solution Concentrate.....	45	Propidium Iodide .....	21
<i>E. coli</i> O45, Positive Control .....	31	Biotinylated Antibodies .....	19, 27	Custom/Bulk Products .....	48
<i>E. coli</i> O103 Antibody.....	31	Blocking Solutions, Enzymes .....	44	Cy Dye-Labeled Conjugates,.....	16, 26
<i>E. coli</i> O103, Positive Control .....	31	Universal Block.....	44	DAB Substrates .....	20
<i>E. coli</i> O111 Antibody.....	31	Blocking Solution Concentrate.....	44	DAB Reagent Set .....	20
<i>E. coli</i> O111, Positive Control .....	31	Blocking Solutions, Protein.....	44	StableDAB Peroxidase Substrate.....	20
<i>E. coli</i> O121 Antibody.....	31	BSA Diluent/Blocking Solution Concentrate.....	44	DAPI.....	21
<i>E. coli</i> O121, Positive Control .....	31	Detector Block.....	44	Detector Block.....	44
<i>E. coli</i> O145 Antibody.....	31	Milk Diluent/Blocking Solution.....	44	Diluent/Blocking Solutions .....	43
<i>E. coli</i> O145, Positive Control .....	31	SignalLOCK.....	44	Blocking Solutions Concentrate .....	44
<i>E. coli</i> O157:H7 Antibody .....	31	Goat Serum.....	44	BSA Diluent/Blocking Solution .....	44
<i>E. coli</i> O157:H7, Positive Control.....	31	Mouse Serum.....	44	Detector Block .....	44
<i>Helicobacter pylori</i> Antibody .....	31	Rabbit Serum.....	44	Milk Diluent/Blocking Solution.....	44
<i>Helicobacter pylori</i> , Positive Control.....	31	Blotting Kits, See HisDetector, and Protein Detector		Normal Serum .....	44
<i>Legionella</i> species Antibody .....	31	His-tagged Protein Western Blotting Kits .....	37	SignalLOCK.....	44
<i>Legionella pneumophila</i> , Positive Control.....	31	Western Blot Kits .....	9	ELISA Microwell Substrates, AP and HRP .....	7
<i>Listeria</i> , genus specific Antibody .....	31	BluePhos Microwell Substrate.....	7	ELISA Microwell Kits, AP and HRP .....	4
<i>Listeria</i> , High Sensitivity Antibody .....	31	BSA Diluent/Blocking Solution Concentrate.....	44	Enzyme-Labeled Conjugates.....	26, 27
<i>Listeria</i> , genus specific, Positive Control.....	31	Buffers/Wash Solutions .....	45	F(ab') <sub>2</sub> Antibodies .....	25
<i>Renibacterium salmoninarum</i> Antibody.....	31	Biotin Wash Solution Concentrate .....	45	Fc Antibodies .....	25
<i>Renibacterium salmoninarum</i> , Positive Control .....	31	Dulbecco's PBST .....	45	FirePhos Microwell AP Substrate System.....	7
<i>Salmonella</i> (CSA-1) Antibody.....	31	PBST.....	45	Fluorochrome Conjugates.....	26, 27
<i>Salmonella typhimurium</i> , Positive Control .....	31	Phosphatase Wash Solution .....	45	Gold-Labeled Conjugates.....	26, 27
<i>Shigella</i> species Antibody .....	31	TBST.....	45	His-tagged Protein Detection .....	37
<i>Shigella</i> , Positive Control.....	31	Wash Solution Concentrate.....	45	HisDetector Nickel Conjugates, AP and HRP .....	37
<i>Vibrio</i> species Antibody.....	31	Bulk and Custom Services.....	49	HisDetector Western Blot Kits.....	37
<i>Vibrio</i> species, Positive Control .....	31	4CN Substrate .....	15	HistoMark Biotin Streptavidin Kits .....	19
<i>Vibrio cholerae</i> Antibody.....	31	Chemiluminescent Detection Kits .....	12	HistoMark Immunohistochemistry Staining Kits .....	20
<i>Yersinia</i> species Antibody .....	31	Chemiluminescent Substrates.....	8, 14	HistoMark HRP Staining Systems.....	20
<i>Yersinia enterocolitica</i> , Positive Control.....	31	LumiGLO HRP .....	8, 14	DAB Reagent Set .....	20
Antibodies and Conjugates		LumiGLO Reserve HRP.....	8, 14	HistoMark BLACK .....	20
Primary Polyclonal, Unlabeled and Labeled		LumiGLO Ultra HRP .....	8, 14	HistoMark ORANGE .....	20
Anti-Bacteria Antibodies & Controls.....	28				

HistoMark TrueBlue .....	20	Peroxidase Conjugates .....	27	Phosphatase .....	8
HistoMark X-Gal Substrate Set .....	20	Peroxidase Substrates .....	7, 14	BCIP/NBT .....	12, 15
StableDAB Peroxidase Substrate .....	20	Phosphatase Conjugates .....	27	Peroxidase .....	12
TrueBlue Substrate .....	20	Phosphatase Substrates .....	7, 14	4CN Substrate System .....	15
HistoMark AP Staining Systems .....	20	Phosphatase Wash Solution .....	45	TMB Membrane Substrates .....	12
HistoMark BLUE .....	20	Phycoerythrin-Labeled Conjugates .....	26	SureBlue TMB Microwell Peroxidase Substrate .....	7
HistoMark RED .....	20	pNPP Microwell Substrate .....	7	SureBlue Reserve TMB Microwell Peroxidase Substrate .....	7
Horseradish Peroxidase-Labeled Conjugates .....	26	Protein Detector Blot Kits .....	12	SureLINK Conjugation Kits, AP and HRP .....	34
Horseradish Peroxidase Substrates .....	6, 10, 14, 15	Protein A Agarose .....	14	TMB BlueStop Solution .....	46
HRP Chemiluminescent Blotting Kit .....	12	Protein G Agarose .....	14	TMB Stop Solution .....	46
HRP Nickel Conjugates, AP and HRP .....	37	Protein Labeling Kits, AP and HRP .....	34	TMB Substrates .....	7, 15
HRP Protein Labeling Kits .....	34	Purification Kits and Reagents .....	39	Membrane Blotting .....	15
HRPStabilizer .....	47	Immunoprecipitation Kits .....	41	Microwell ELISA .....	7
Immunohistochemical Kits and Substrates .....	17	Immunopurification Kits .....	41	TrueBlue Substrate .....	20
HistoMark X-Gal Substrate Set .....	20	Protein A Agarose .....	41	Universal Block for Immunohistochemistry .....	44
Peroxidase .....	20	Protein G Agarose .....	41	Unlabeled Antibodies .....	27
DAB Reagent Set .....	20	R-Phycoerythrin-Labeled Antibodies .....	26	Wash Solutions .....	45
HistoMark BLACK .....	20	Rhodamine-Labeled Antibodies .....	26	Biotin Wash Solution Concentrate .....	45
HistoMark ORANGE .....	20	SignalOCK HRP ChemiWestern (Film) .....	12	D-PBST .....	45
HistoMark TrueBlue .....	20	SignalOCK HRP ChemiWestern (Imager) .....	12	PBST .....	45
StableDAB Peroxidase Substrate .....	20	SignalOCK AP ChemiWestern (Film/Imager) .....	12	Phosphatase Wash .....	45
TrueBlue Substrate .....	20	Stabilizers, Conjugate, AP and HRP .....	47	TBST with Tween .....	45
Phosphatase .....	20	StableDAB Peroxidase Substrate .....	20	Wash Solution Concentrate .....	45
HistoMark BLUE .....	20	Streptavidin Conjugates .....	19	Western Blotting Reagents and Kits .....	9
HistoMark RED .....	20	STEC Antibodies .....	31	Western Blotting Substrates .....	12
Immunoprecipitation Kits .....	41	Stop Solutions .....	46	X-Gal Substrate Set .....	20
Protein A Agarose .....	41	ABTS Stop Solution .....	46		
Protein G Agarose .....	41	APStop Solution .....	46		
Immunopurification Kits .....	41	TMB BlueStop Solution .....	46		
Industrial/Custom Products .....	49	TMB Stop Solution .....	46		
LumiGLO Chemiluminescent Substrate .....	14	Substrates, Chemiluminescent .....	8, 14		
LumiGLO Reserve Chemiluminescent Substrate .....	14	LumiGLO HRP Chemiluminescent Substrate .....	8, 14		
LumiGLO Ultra Chemiluminescent Substrate .....	14	LumiGLO Reserve HRP Chemiluminescent Substrate .....	8, 14		
Membrane Assay Kits .....	9	LumiGLO Ultra Chemiluminescent Substrate .....	14		
Chemiluminescent Blotting Kits .....	12	PhosphaGLO AP Substrate .....	14		
HisDetector Western Blot Kits .....	37	PhosphaGLO Reserve AP Substrate .....	14		
Protein Detector Blotting Kits .....	12	Substrates, Colorimetric .....	7, 15		
SignalOCK HRP ChemiWestern (Film) .....	12	Microwell ELISA .....	7		
SignalOCK HRP ChemiWestern (Imager) .....	12	Phosphatase .....	7		
SignalOCK AP ChemiWestern (Film/Imager) .....	12	BluePhos Microwell Substrate .....	7		
Microwell ELISA Assay Kits .....	4	pNPP Substrate .....	7		
Microwell ELISA Substrates .....	7, 8	FirePhos Microwell Phosphatase Substrate .....	7		
Milk Diluent/Blocking Solution Concentrate .....	44	Peroxidase .....	7		
Nickel Conjugates, AP and HRP .....	37	ABTS Substrate .....	7		
Normal Goat Serum .....	44	SureBlue TMB Substrate .....	7		
Normal Mouse Serum .....	44	SureBlue Reserve TMB Substrate .....	7		
Normal Rabbit Serum .....	44	TMB Microwell Substrates .....	7		
OEM Products .....	49	Membrane Blotting .....	8		
Orcein Counterstain .....	21				



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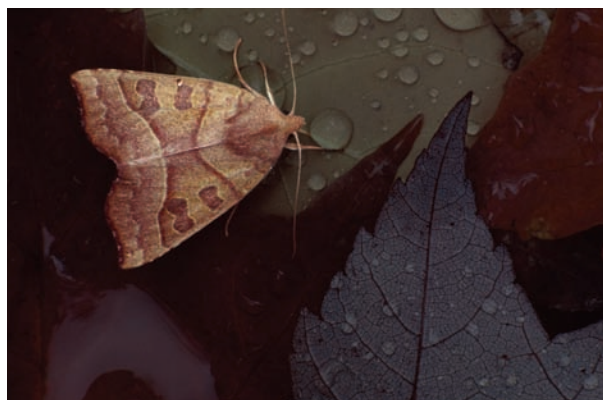
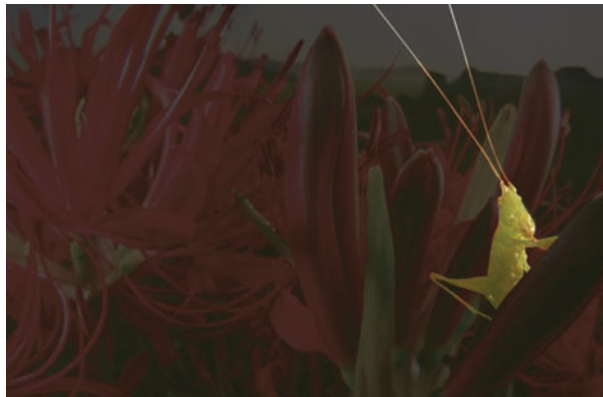
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